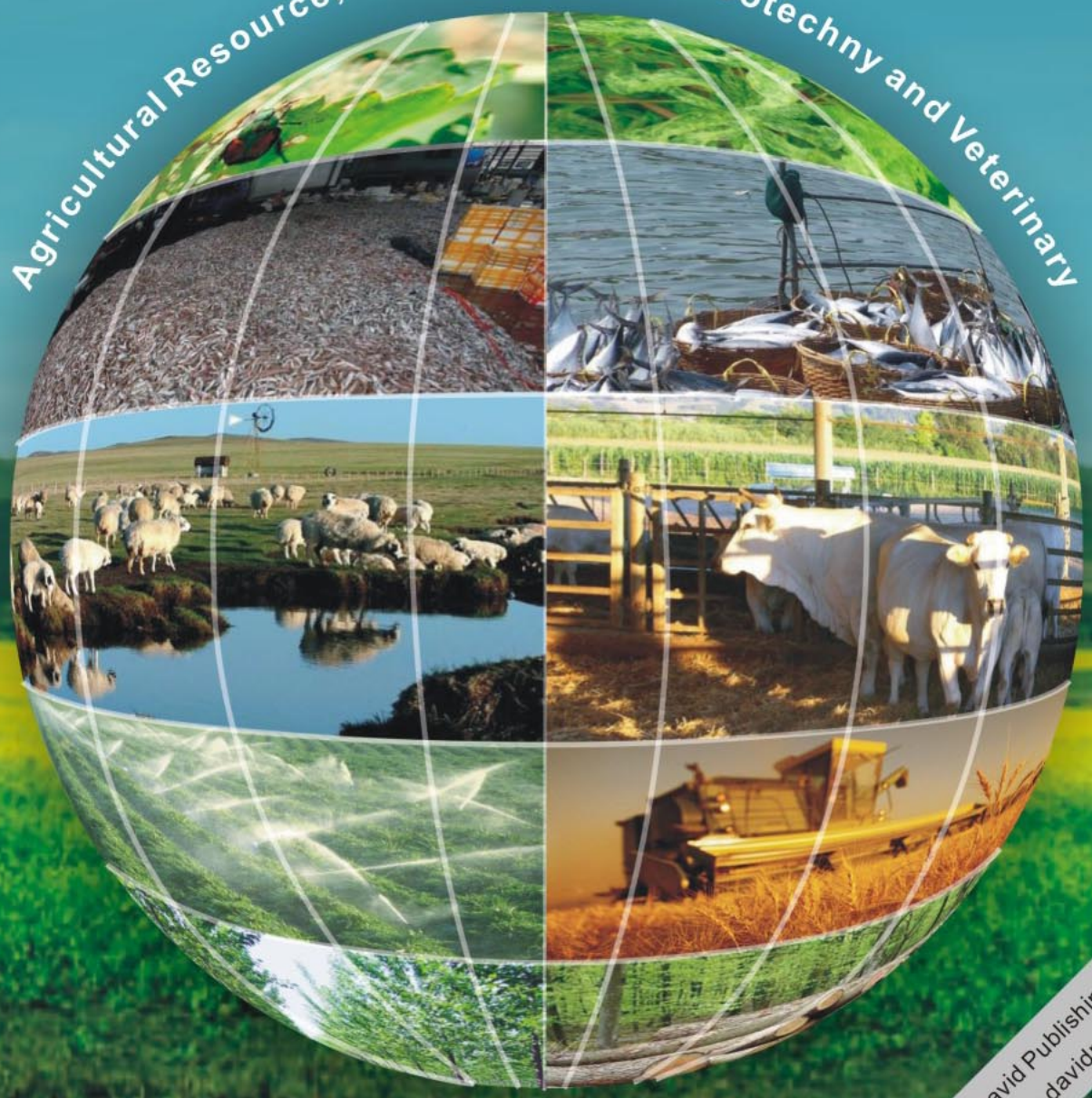
DAVID PUBLISHING

From Knowledge to Wisdom

ISSN 2161-6256

Journal of Agricultural Science and Technology A

Volume 4, Number 2, February 2014



David Publishing Company
www.davidpublishing.com

Journal of Agricultural Science and Technology A

Volume 4, Number 2, February 2014 (Serial Number 34)

David Publishing Company
www.davidpublishing.com

Publication Information:
Journal of Agricultural Science and Technology A (Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250) is published monthly in hard copy (ISSN 2161-6256) by David Publishing Company located at 240 Nagle Avenue #15C, New York, NY 10034, USA.

Aims and Scope:
Journal of Agricultural Science and Technology A, a monthly professional academic journal, particularly emphasizes new research results in agricultural resource, plant protection, zootechny and veterinary, all aspects of animal physiology, modeling of animal systems, agriculture engineering and so on. Articles interpreting practical application of up-to-date technology are also welcome.

Manuscripts and correspondence are invited for publication. You can submit your papers via web submission, or E-mail to agriculture@davidpublishing.org. Submission guidelines and web submission system are available at http://www.davidpublishing.org, http://www.davidpublishing.com.

Editorial Office:
240 Nagle Avenue #15C, New York, NY 10034, USA
Tel: 1-323-984-7526, 323-410-1082
Fax: 1-323-984-7374, 323-908-0457
E-mail: agriculture@davidpublishing.org, agriculture66@hotmail.com, agriculture@davidpublishing.com

Abstracted/Indexed in:
Database of EBSCO, Massachusetts, USA
Chemical Abstracts Service (CAS), USA
Cambridge Scientific Abstracts (CSA), ProQuest Science Journals, USA
Ulrich's Periodicals Directory, USA
Summon Serials Solutions, USA
Chinese Database of CEPS, American Federal Computer Library Center (OCLC), USA
China National Knowledge Infrastructure (CNKI), China
Chinese Scientific Journals Database, VIP Corporation, Chongqing, China
Norwegian Social Science Data Services (NSD), Norway
Index Copernicus, Poland
Universe Digital Library Sdn Bhd (UDLSB), Malaysia
Google Scholar

Subscription Information:
Price (per year)
Print $1200

David Publishing Company
240 Nagle Avenue #15C, New York, NY 10034, USA
Tel: 1-323-984-7526, 323-410-1082 Fax: 1-323-984-7374, 323-908-0457
E-mail: order@davidpublishing.com
Digital Cooperative Company: www.bookan.com.cn

David Publishing Company
www.davidpublishing.com

Journal of Agricultural Science and Technology A

Volume 4, Number 2, February 2014 (Serial Number 34)

Contents

Journal of Agricultural Science and Technology A 4 (2014) 103-111
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Utilization of Fungi for the Biological Control of Insect Pests and *Ganoderma* Disease in the Indonesian Oil Palm Industry

Hari Priwiratama and Agus Susanto

Crop Protection Division, Indonesian Oil Palm Research Institute, Medan 20158, Indonesia

Received: October 30, 2013 / Published: February 20, 2014.

Abstract: Biological control is usually the first choice of control and prevention method for integrated pest management (IPM) strategies and has now been widely implemented by Indonesian oil palm plantations. Entomopathogenic fungus, i.e., *Metarhizium anisopliae*, *Cordyceps militaris* and *Beauveria bassiana* have been demonstrated to control renowned pests of oil palm. *Metarhizium* has been used to control *Oryctes* larvae and the mortality has ranged from 91.67% to 100% in laboratory and 7.4% to 88.75% in the field. *Metarhizium* has been applied in combination with a termite baiting system (TBS) to control termites in the field for preventive and curative action as well. In many oil palm plantations in Indonesia, *Cordyceps* has been used to reduce the field moth population of *Setothosea asigna*. Application of *Cordyceps* within the oil palm circle was able to infect *S. asigna* pupae up to 80%. Meanwhile, *Beauveria* in an effervescent formulation was demonstrated to have better efficacy on *Darna trima* larvae. A significant finding on the biological control of basal stem rot disease (*Ganoderma*) was the isolation of *Trichoderma* sp. and *Gliocladium* sp.. The efficacy was conducted with promising result and techniques on the application of *Trichoderma* have been developed, i.e., hole-in-hole system, surgery and a mounding method. However, as the roots developed, *Trichoderma* was no longer able to protect the palm from *Ganoderma*. In spite of that, the use of *Trichoderma* still prolonged the life of oil palms by up to 2-3 years. Another fungi belonging to vesicular arbuscular mychorrhiza (VAM) has been developed to control *Ganoderma*. The efficacy in the nursery showed promising results and the *Ganoderma* incidence remained low compared to the untreated control. Large scale field trials are ongoing. Challenges on the implementation of biological control in oil palm plantations are because of application and availability of biopesticides/natural enemies. Therefore, advances in research on the formulation of biological control agents are still needed.

Key words: Entomopathogenic fungus, biological control, mixture formula, *Ganoderma*, VAM.

1. Introduction

Infestation by insect pests, particularly leaf-eating Lepidoptera, may significantly reduce oil palm fresh fruit bunch production (FFB). Defoliation of 50% of fronds by the infestation of leaf caterpillars will cause 30%-40% yield loss of eight-year old oil palm for two years after defoliation [1]. On the other hand, the presence of basal stem rot (BSR) disease caused by *Ganoderma boninense* was reported to cause 50%-80% loss on the number of stand trees per hectare in some oil palm plantations in Indonesia which led to further severe FFB losses [2, 3]. Pest infestation and disease infection are limiting factors in oil palm cultivation.

For many years, continuous application of chemical insecticides has commonly been practiced to control insect pest populations [4]. This has disrupted the dynamics of the natural population of pest predators and increased the risk of pest resistance and resurgence [5]. Efforts to control BSR disease using chemical fungicides was demonstrated with no promising results and will be potentially

Corresponding author: Hari Priwiratama, research fields: biological control of pests and diseases, etiology of oil palm disease, field management of pests, diseases and weeds. E-mail: hari.priwira@iopri.org; hari.priwira@gmail.com.

environmentally dangerous [3].

Increase of the awareness of many issues due to continuous application of pesticides for controlling pests and diseases has accelerated the development of biological control [6, 7]. Recently, the Indonesia government has formulated the Indonesia Sustainable Palm Oil (ISPO) regulations to be mandatory implemented by the industry. The ISPO regulations have now led the oil palm industry to adopt the integrated pest management (IPM) methodologies and biological control is often the first choice to manage pest infestations and disease infections. This will greatly increase the future role of biological agents in oil palm.

Over decades, the use of viruses, bacteria, predators, parasitoids, nematodes and fungi has been demonstrated to manage pests and diseases in oil palm plantation. Among these, fungi are the most popular because of greater efficacy with wider host range and reproducibility on larger scale. This paper will further discuss the progress on the use of fungi for the biological control of insect pests and *Ganoderma* disease in the Indonesian oil palm industry.

2. Fungi for Controlling Oil Palm Insect Pests

Utilization of entomopathogenic fungi to control insect pests has widely been implemented in oil palm plantations. *Metarhizium anisopliae*, *Beauveria bassiana* and *Cordyceps militaris* are common fungi used as bio-control agents to manage populations of insect pests in oil palm plantations [8-12]. Efficacy of each fungus has been demonstrated with promising results in green houses as well as in the field.

2.1 Metarhizium anisopliae

Green muscardine fungus, *M. anisopliae*, was reported to have a wide host range [13]. The use of *M. anisopliae* to control *O. rhinoceros* larvae was evaluated in 1970s [14]. Among many isolates tested, *M. anisopliae* var. *major* was found to have higher virulency against *Oryctes* beetles [10]. Since 1980s, efforts to mass propagate *M. anisopliae* for field testing have been initiated in Indonesia [15].

Pathogenicity tests with various formulations of *M. anisopliae* on *Oryctes rhinoceros* larvae in the laboratory usually produced promising results [16-18]. However, its application in the field was not always as good as in the laboratory. Large scale application of *M. anisopliae* to control *O. rhinoceros* was conducted in Asahan region of North Sumatra [17]. Two types formulation of *M. anisopliae* was applied onto oil palm empty fruit bunches (EFB) with a rate of 20 g/m^2. Results showed that higher mortality of *O. rhinoceros* larvae were observed on the application of a powder formulation (Fig. 1). The incubation period of *M. anisopliae* in experiments was approximately 10-14 days after application. Mortality of *O. rhinoceros* larvae continuously increased until six weeks after application. Better coverage of powder formulation on the empty fruit bunch is likely to be one of the factors causing higher percentage of infected larvae. Infectivity of the *M. anisopliae*, however, only lasted approximately 4-6 months after formulation.

Another field experiment was conducted in Teluk Dalam Estate in Asahan region [16]. The use of 100 g of *M. anisopliae* to reduce the population of *O. rhinoceros* larvae in the big hole planting system, in which oil palm seedlings planted in a 3 × 3 × 1 m^3 planting hole with addition of three layers EFB, was been evaluated. Results showed that average mortality of *O. rhinoceros* larvae is continuously increased on both plots until seven weeks after application (WAA) (Table 1). The use of *M. anisopliae* gave 75% mortality of *O. rhinoceros* larvae compared to control treatment. Natural infection of *M. anisopliae* was also found in Teluk Dalam Estate, since infection was observed in the control treatment.

Reduction in field populations of *O. rhinoceros* larvae after *M. anisopliae* application was also observed in Sei Mangke Estate. It was reported that larvae population on EFB in big hole planting system reduced from 231.5 individuals per sampling plot (ISP)

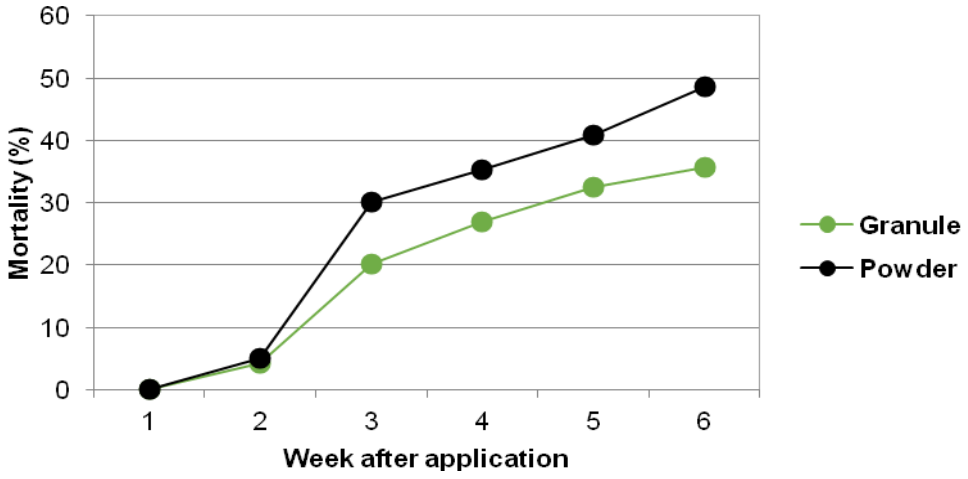


Fig. 1 Mortality of *O. rhinoceros* larvae after application of two different formula of *M. anisopliae*.

Table 1 Percentage of *O. rhinoceros* larvae infected by *M. anisopliae* in big hole planting system in Teluk Dalam Estate.

Treatment	Mortality (%)		
	2 WAA	5 WAA	7 WAA
M. anisopliae of 100 g/hole	24.05	46.70	75.00
Control	6.40	9.13	15.79

at 2 WAA to 103 ISP at 7 WAA (Fig. 2). Results showed that the application of *M. anisopliae* is essential to reduce *O. rhinoceros* population, particularly in estates which implementing EFB application in the field.

2.2 Beauveria bassiana

White muscardine fungus, *B. bassiana*, is very popular in horticultural crops with wide host range, i.e., aphids, thrips, flies, beetles, nettle caterpillar, ants and termites [19-23]. Despite isolates of *B. bassiana* being available, application in large commercial oil palm plantations in Indonesia has never been reported.

2.3 Cordyceps militaris

Cordyceps militaris was known to have high pathogenicity to nettle caterpillar pupae [24, 25]. Mass production and field use of *C. militaris* in oil palm plantation in Indonesia has been started since 1990s [26, 27]. Early result of *C. militaris* application using solid substrate has suggested that *C. militaris* was able to reduce *Setothosea asigna* pupae population of which the infection rate increased from 46.1% to 80.5% [28]. It initiated intensive research on the utilization of *C. militaris* in the field. However, hand picking of *S. asigna* pupae is more popular than application of *C. militaris*, and therefore has rarely been reported [29].

2.4 Recent Formulation for Commercial Use

Over decades, basic fermentation technology to mass produce fungi followed by mixing the spores or mycelium onto various formulations, i.e., powder, granule and oil, has been widely practiced to commercialise the products [17, 30-33]. In some plantations, application of fungus and virus by simply mixing infected larvae with water became the most common practice for controlling the pests [34]. Despite the high pathogenicity it has been rarely adopted because of rapid decreasing viability and pathogenicity, high volumes required for large scale application, high costs of distribution and inefficiency issues [35].

For ease of use in the field, tripartite collaboration between Indonesian Oil Palm Research Institute (IOPRI), Indonesian Sugar Research Institute (ISRI) and Prima Agro Tech, Ltd., was initiated to produce a mixture of entomopathogenic agents consisting of *M.*

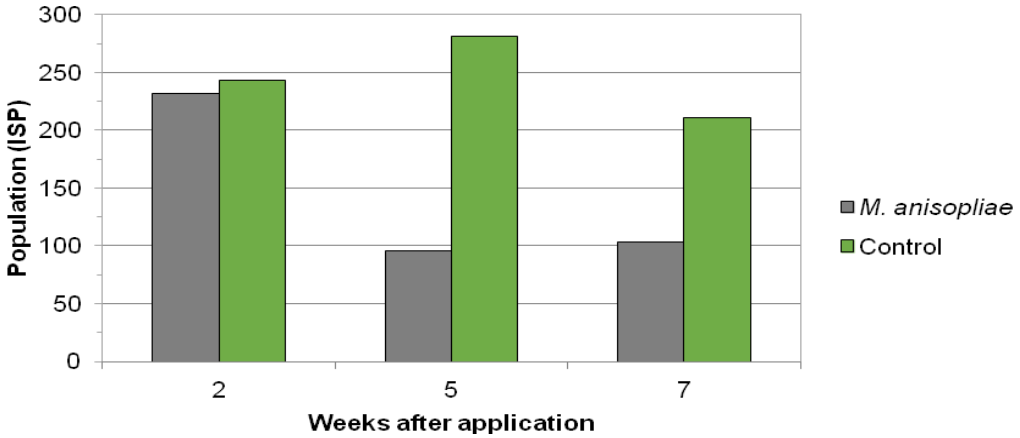


Fig. 2 Population of *O. rhinoceros* after *M. anisopliae* application in Sei Mangke Estate.

anisopliae, *C. militaris*, *Beauveria bassiana* and *B. thuringiensis* formulated as an effervescent tablet, known as Metarizep [35]. Preliminary observations showed that the entomopathogenic agents were able to survive in the effervescent formula and further pathogenicity tests were conducted in green houses and in the field using a rate one tablet per four litres of water.

Green house efficacy to *Oryctes* larvae showed that infection of *M. anisopliae* was observed with all effervescent treatments at seven days after application (DAA) compared to 10 DAA with a maize-granule formulation (Fig. 3). Higher mortality of larvae at seven DAA and 10 DAA was shown on effervescent containing only *M. anisopliae*, and at 14 DAA, mortality of larvae was found at the same level for all treatments. The results suggest that *M. anisopliae* in effervescent formula was more effective than the standard formulation and there was no antagonistic effect in the formulation. Field efficacy of the formula, however, showed lower mortality than in the green house and ranged from 7.4% to 26% for effervescent tablets with only *M. anisopliae* and 8.9%-44.7% for the mixture, whilst 16.7%-36.1% mortality with longer incubation period being observed with standard formula application.

Promising results with the use of the effervescent mixture to nettle caterpillar have also been reported [35]. 100% mortality of *Darna trima* was observed with a *Beauveria*-based effervescent and its mixture-effervescent formula at three DAA of which shown by the development of *B. bassiana* spores on larvae's cuticle (Fig. 4a). Another patoghenicity test to *S. asigna* pupae showed that initial development of *C. militaris* ascospores on *S. asigna* cocoons was determined at 36 DAA on effervescent treatments (Fig. 4b) and mortality of pupae ranged from 40.0% to 43.3% at the end of observation (55 DAA). Unfortunately, no mortality of *M. corbetti* and less than 10% mortality of *M. plana* were observed during the preliminary test.

Application of the mixture formulation in a termite baiting system (TBS) using wasted cardboard was demonstrated that a combination of TBS and mixture formulation was able to decrease termite's infestation intensity from 60% at 15 DAA to 0% at 45 DAA [35]. Termite infected by *M. anisopliae* was observed during the observation (Fig. 4c). Effervescent formulation containing Multiple Nucleo Polyhedra Virus (MNPV), a type of virus infecting nettle caterpillars, recently has been also produced and field efficacy of the formula to *S. asigna* is being conducted.

3. Fungi for Controlling Basal Stem Rot Disease

Until now, BSR disease caused by *Ganoderma boninense* remains the only devastating disease of oil

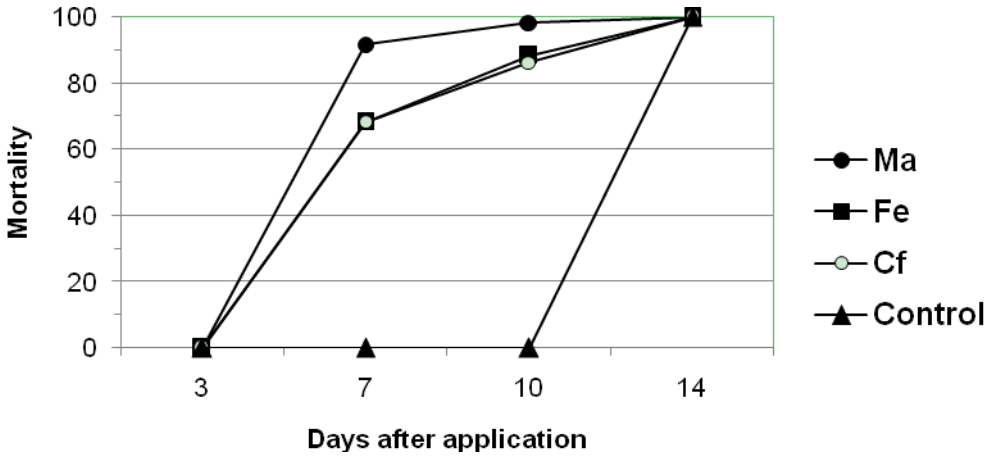


Fig. 3 Mortality of *Oryctes* larvae with several effervescent agents.
Ma: effervescent of *M. anisopliae*; Fe: effervescent of *M. anisopliae*, *C. militaris* and *B. bassiana*; Cf: effervescent of fungus and *B. thuringiensis*; Control: granule formulation of *M. anisopliae*.

Fig. 4 Infected insect pest on the application of consortium entomopathogenic fungus.
(a) Sporulation of *Beauveria* on *D. trima*; (b) initial ascospore of *Cordyceps* development on *S. asigna* cocoon; (c) termite infected by *Metarhizium*.

palm in Indonesia [3]. In 1980s, investigations were initiated to find superior biological control agents from the oil palm rhizosphere. A significant finding was the isolation of *Trichoderma* sp. and *Gliocladium* sp. of which are common fungus used to control *Ganoderma* [36-38]. Efficacy was conducted with promising result and in 2000s, mass production of *Trichoderma* was initiated and distributed to many oil palm plantations in Indonesia.

3.1 Trichoderma spp.

Trichoderma spp. and *Gliocladium* spp. were tested *in vitro* and *in vivo* to suppress *G. boninense*, and both agents have shown promising results [39-42]. In 2000s, *Trichoderma koningii* has been successfully formulated and became popular to control BSR on oil palm in Indonesia [43]. Bipartite collaboration between IOPRI and PT. Bio Industri Nusantara has formulated a biofungicide containing *Trichoderma koningii* and *Trichoderma harzianum* to control *Ganoderma*. Approximately 10,000 ha of oil palm plantation have been treated with *Trichoderma* sp. every year by application to planting holes.

The results of efficacy test in the nursery showed that BSR disease incidence on *Trichoderma* application is significantly lower than control treatment. After seven months of treatment, incidence of *Ganoderma* on oil palms treated solely with *Trichoderma* is only 1% compared to 30% on control treatment. Results of field study show that application of *Trichoderma* should be combined with hole-in-hole technique, of which conducted by planting the

seedling in a planting hole ($0.6 \times 0.6 \times 0.6$ m^3) inside the big hole planting system, to better reduce incidence of disease caused by *Ganoderma* [44, 45]. However, as the roots develop, *Trichoderma* is no longer able to protect the palm from *Ganoderma.* In spite of that, the use of *Trichoderma* is still needed to prolong the life of oil palm by up to 2-3 years.

3.2 Vesicular Arbuscular Mycorrhizae (VAM)

Another potential soil-borne agent to control *G. boninense* is mycorrhizal arbuscular fungus, which have ability to follow the development of the roots [46]. Mycorrhizae protect plants from pathogen attack through nutrient competition with pathogens, including the production of siderophores and protective layer, and induction of host defense mechanism [47, 48].

Efficacy of mycorrhizae to control BSR disease was demonstrated in the nursery [49]. The results showed that lower incidence of BSR disease was observed on seedlings treated with mycorrhizae (Fig. 5). Application of 50 g and 70 g of mycorrhiza was able to prolong the incubation period of *Ganoderma*. These results suggest that mycorrhizae can be used as a biological agent to control *Ganoderma* disease. Thereafter, IOPRI and The Assessment Biotechnology Center, Agency for the Assessment and Application of Technology (BPPT) have been working together reviewing and producing a product containing VAM (*Gigasporaceae* sp., *Acaulospora* sp. and *Glomus* sp.) and plant growth promoting rhizobacteria (PGPR) (*Azotobacter* sp., *Bacillus* sp., *Pseudomonas* sp. and *Corynebacterium* sp.) and *Trichoderma harzianum*, known as Mycorix Plus.

Incidence of BSR disease on the application of 50, 100 and 150 g of Mycorix Plus was 6.25%, 4.68% and 1.56%, respectively, whilst positive control 3.13% (Fig. 6). The result indicated that the increasing rates of Mycorix Plus can reduce the disease incidence of *Ganoderma*. It was also demonstrated that timing for application will determine the success of biological control of BSR disease. Application of Mycorix Plus three months before inoculation of *Ganoderma* provided an opportunity for the mycorrhizae to colonize the root tissues of which resulting on lower *Ganoderma* infection. Research to evaluate field efficacy of the product is ongoing.

4. Conclusions

The efficacy results of antagonistic fungi on various insect pests and *Ganoderma* disease of oil palm have clearly shown the potential use of fungi on pests and diseases management. Development in the formulation of fungi by mixing various antagonistic fungi in one simple formulation has improved its ease of use as well as its efficacy in the field. However, further evaluation in large scale oil palm field trials is still essential.

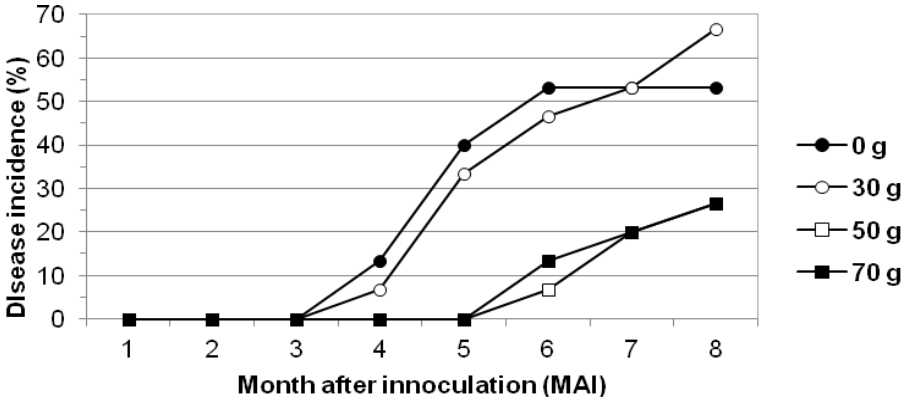


Fig. 5 Development of BSR disease incidence on the application of mycorrhizae in the oil palm nursery.

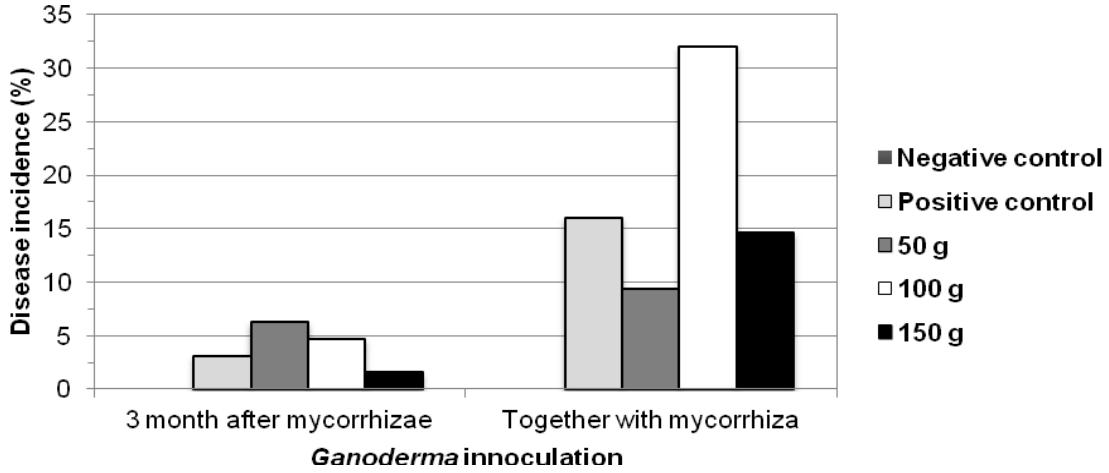


Fig. 6 Effect of doses and timing of Micorix Plus application on BSR disease incidence in oil palm nursery.

Acknowledgments

The authors thank Dr. Stephen Peter Connor Nelson for valuable assistance in paper improvements.

References

[1] B.J. Wood, R.H.V. Corley, K.H. Goh, Studies on the effect of pest damage on oil palm yield, in: Advanced in Oil Palm Cultivation, R.L. Wastrie, D.A. Earp (Eds.), The Incorp. Soc. of Plant, 1972, pp. 360-379.

[2] A. Subagio, H.L. Foster, Implications of *Ganoderma* disease on loss in stand and yield production of oil palm in North Sumatra, in: Proceedings of the 6th International Conference on Plant Protection in the Tropics, Kuala Lumpur, 2003.

[3] A. Susanto, *Ganoderma* in oil palm plantation time after time, in: Proceedings of National Symposium and Workshop of *Ganoderma*, 2011. (in Bahasa)

[4] R.H.V. Corley, P.B. Tinker, The Oil Palm, 2nd ed., Blackwell Science, Oxford, 2003.

[5] S. Prawirosukarto, L. Pane, A. Wahyu, T. Liwang, The implementation of IPM on the management of nettle caterpillars and bagworms in Sinar Mas Group oil palm plantation: Optimizing the use and conservation of natural enemies, in: Proceedings of the Technical Meeting for Oil Palm Cultivation, 2005. (in Bahasa)

[6] A. Darus, M.B. Wahid, Intensive IPM for management of oil palm pests, Oil Palm Bulletin, 2000.

[7] G.D. Inglis, M.S. Goettel, T.M. Butt, H. Strasser, Use of hyphomycetous fungi for managing insect pests, in: T.M. Butt, C. Jackson, N. Magan (Eds.), Fungi as Biocontrol Agents: Progress, Problems and Potential, 2001, pp. 23-70.

[8] A.S. Ramlah, M.W. Basri, M. Ramlee, Pathogenicity test of *Beauveria bassiana* (Balsamo) against oil palm bagworm (*Metisa plana* Wlk.), Elaeis 5 (1993) 92-101.

[9] S. Prawirosukarto, A. Djamin, D. Pardede, Integrated pest management of leaf eating caterpillar, in: Proceedings of the Technical Meeting for Oil Palm Cultivation, 1997, pp. 33-47. (in Bahasa)

[10] M. Ramle, M.W. Basri, H.K. Norman, A.A.S. Ramlah, Pathogenicity of four isolates of *M. anisopliae* on rhinoceros beetles, *Oryctes rhinoceros* and pollinating weevil, *Elaeidobius kamerunicus* and its DNA profiles revealed by RAPD-PCR, in: Proceedings of the PORIM International Palm Oil Conference, 1999, pp. 207-219.

[11] A. Susanto, S. Prawirosukarto, F. Yanti, Conservation and mass production of natural enemies, IOPRI Book Series, 2005, pp. 15-18. (in Bahasa)

[12] D. Soetopo, I.G.A.A. Indrayani, Current technology and prospect of *Beauveria bassiana* on the management of insect pests in perennial crops, Prespektif 6 (2007) 29-46. (in Bahasa)

[13] I. Stolz, The effect of *Metarhizium anisopliae* (Metsch.) Sorokin (Flavoviridae) Gams and Rozypal var. *acridum* (Deuteromycotina: Hyphomycetes) on non-target Hymenoptera, Ph.D. Dissertation, Faculty of Science, University of Basel, 1999.

[14] G.C.M. Latch, R.E. Falloon, Studies on the use of *Metarhizium anisopliae* to control *Oryctes rhinoceros*, Entomophaga 21 (1976) 39-48.

[15] D. Sitepu, S. Kharie, J.S. Waroka, H.F.J. Motulo, Methods for the production and use of *Metarhizium anisopliae* against *Oryctes rhinoceros*, in: Integrated Coconut Pest Control Project Report, Coconut Research Institute, 1988, pp. 104-111.

[16] A. Susanto, A.P. Dongoran, F. Yanti, A.F. Lubis, A.E. Prasetyo, The role of *Metarhizium anisopliae* on reducing of *Oryctes rhinoceros* larvae in empty fruit bunch of oil

palm mulch, in: Proceedings of the International Oil Palm Conference, 2006.

[17] A. Susanto, A.P. Dongoran, F. Yanti, The improvement of *Metarhizium anisopliae* to enhance it's pathogenicity, in: Proceedings of the PIPOC International Oil Palm Congress, 2007, pp. 729-736.

[18] H. Priwiratama, E.M. Manurung, A. Susanto, Effectivity of oil based formulation of *M. anisopliae* on the mortality of *Oryctes rhinoceros* larvae, in: Proceedings of the Fourth IOPRI-MPOB International Seminar: Existing and Emerging of Pests and Diseases of Oil Palm—Advances in Research and Management, 2012.

[19] C. Wang, J.E. Powell, Isolation and evaluation of *Beauveria bassiana* for control of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae), Sociobiology 41 (2002) 1-13.

[20] F. Posada, F.E. Vega, Establishment of the enthomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*), Mycologia 97 (2005) 1195-1200.

[21] M.S. Al Mazrawi, Impact of the enthomopathogenic fungus *Beauveria bassiana* on the honey bees *Apis mellifera* (Hymenoptera: Apidae), World Journal of Agricultural Science 3 (2007) 7-11.

[22] M. Sodiq, D. Martiningsia, Effect of *Beauveria bassiana*on the mortality of *Oecophylla smaragdina* (Hymenoptera: Formicidae), J. Entomol. Indon. 6 (2009) 53-59. (in Bahasa)

[23] S. Sucipto, L.R. Adawiyah, Effectiveness of entomopathogenic fungus *Beauveria bassiana*as biological control agent of *Crocidolomia binotalis* and its correlation to the growth and productivity of *Brasica juncea*, Embryo 8 (2011) 65-72. (in Bahasa)

[24] H.C. Evans, Fungal pathogens of Limacodidae, in: Slug and Nettle Caterpillars, CAB Inter., 1987, pp. 207-212.

[25] R. de Chenon, S. Prawirosukarto, A. Sipayung, Prospects in the use of a fungal pathogen from the genus *Cordyceps* (Ascomycotina) for microbiological control of nettle caterpillars (Limacodidae), in: Proceedings of the First Entomology Society of Indonesia Congress, 1990, p. 10.

[26] B. Papierok, R. de Chenon, J.M. Freulard, W.P. Suwandi, New perspectives in the use of a *Cordyceps* fungus in the biological control of nettle caterpillars in oil palm plantations, in: Proceedings of the PIPOC International Palm Oil Congress, 1993.

[27] H. Wibowo, A. Sipayung, R.D. de Chenon, Mass propagation technique of *Cordyceps* sp. to control *Setothosea asigna* Moore (Lepidoptera: Limacodidae), Bul. PPKS 2 (1994) 147-154. (in Bahasa)

[28] D. Pardede, C.U. Ginting, H. Wibowo, Integrated pest management of nettle caterpillar *Setothosea asigna* Van Eecke in oil palm plantation, Annual report PPKS-AP2I, 1996, pp. 1-19. (in Bahasa)

[29] A. Susanto, A.E. Prasetyo, D. Simanjuntak, T.A.P. Rozziansha, H. Priwiratama, S. Prawirosukarto, et al., Early warning system: Nettle caterpillar, leaf caterpillar and bagworm, Indonesian Oil Palm Research Institute, Medan, 2012. (in Bahasa)

[30] S. Prawirosukarto, A. Susanto, Z.A. Harahap, E. Purnomo, Management of *Oryctes rhinoceros* in the empty fruit bunch, in: Prosiding Pertemuan Teknis Kelapa Sawit, 2000, pp. 51-61. (in Bahasa)

[31] M. Ramle, K. Norman, B.N. Ang, S.A.A. Ramlah, M.W. Basri, Application of powder formulation of *Metarhizium anisopliae* to control *Oryctes rhinoceros* in rotting oil palm residues under leguminous cover crops, Journal of Oil Palm Research 19 (2007) 318-330.

[32] M. Ramle, H.K. Norman, M.W. Basri, Pathogenicity of granule formulations of *Metarhizium anisopliae* against the larvae of the oil palm rhinoceros beetle, *Oryctes rhinoceros* L., Journal of Oil Palm Research 21 (2009) 602-612.

[33] E.M. Manurung, M.C. Tobing, L. Lubis, H. Priwiratama, Efficacy of various *Metarhizium anisopliae* formulation on the mortality of *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) larvae in the insectariums, Jurnal Agroteknoekologi 1 (2013) 47-63. (in Bahasa)

[34] A. Susanto, A.E. Prasetyo, S. Prawirosukarto, H. Priwiratama, T.A.P. Rozziansha, Integrated pest management of *Oryctes rhinoceros* in oil palm plantation, Indonesian Oil Palm Research Institute, Medan, 2012.

[35] A. Susanto, W.E. Widayati, H. Priwiratama, T.A.P. Rozziansha, D. Simanjuntak, A.E. Prasetyo, Consortium of entomopathogenic for controlling insect pests in oil palm plantation, in: Prosiding Pertemuan Teknis Kelapa Sawit, 2013. (in Bahasa)

[36] A.S. Idris, Basal stem rot in Malaysia: Biology, economic importance, epidemiology, detection and control, in: Proceedings of the International Workshop of Awareness, Detection and Control of Oil Palm Devastating Diseases, 2009.

[37] A. Susanto, S. Prawirosukarto, R.Y. Purba, Biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantation, in: Proceedings of the Third International Workshop On *Ganoderma* Diseases of Perennial Crops, 2003.

[38] A. Susanto, L.K. Huan, Management of *Ganoderma* in mineral and peat soil in Indonesia, in: Proceedings of the Second International Seminar Oil Palm Diseases—Advances in *Ganoderma* Research and Management, 2010.

[39] A.L. Abadi, Biology of *Ganoderma boninense* Pat. on oil palm (*Elaeis guineensis* Jacq.) and effect of antagonist soil microbes on its growth, Faculty of Agriculture, IPB,

1987. (in Bahasa)

[40] O.S. Dharmaputra, Antagonistic fungi of *Ganoderma boninense* Pat., the causal agent of basal stem rot disease on oil palm in Adolina Estate, North Sumatera, Annual Research Report P. P. Marihat-Biotrop, 1989. (in Bahasa)

[41] H. Hadiwiyono, M.S. Sinaga, B. Tjahjono, I. Anas, Evaluation on the virulency of *Trichoderma viride*, *Gliocladium fimbriatum* and fluorescence *Pseudomonas* as the natural enemies to control *Ganoderma boninense* Pat. on rubber wood blocks, Buletin Hama & Penyakit Tumbuhan 9 (1997) 26-31. (in Bahasa)

[42] F. Abdullah, G.N.M Ilias, Application *Trichoderma harzianum* in the control of basal stem rot of oil palms, Journal of Zhejiang University (Agric. & Life Sci.) 30 (2004) 391.

[43] H. Soepena, R.Y. Purba, S. Prawirosukarto, A Control Strategy for Basal Stem Rot (*Ganoderma*) on Oil Palm, CABI Publishing, 2000.

[44] A. Susanto, R.Y. Purba, S.M. Purba, A.E. Prasetyo, Development of basal stem rot disease (*Ganoderma boninense*) in big hole planting system, in: Proceedings of the International Oil Palm Conference, 2006.

[45] H. Widiastuti, S. Suharyanto, A. Susanto, S. Sugiyono, R. Rais, B. Budi, Assessment of the effectiveness of AM fungal as active agent of biofertilizer and in combination with *Trichoderma* as biocontrol for oil palm seedling under commercial scale production, in: Proceedings of the International Oil Palm Conference, 2006.

[46] D.J. Read, D.H. Lewis, A.H. Fitter, I.J. Alexander, Mycorrhizas in Ecosystems, Cab International, 1992.

[47] L.L. Valentine, T.L. Fielder, A.N. Hart, C.A. Petersen, H.K. Berninghausen, D. Southworth, Diversity of ectomycorrhizas associated with *Quercus garryana* in Southern Oregon, Canadian Journal of Botany 82 (2004) 123-135.

[48] M.C. Brundrett, Coevolution of roots and mycorrhizas of land plants, Newphytologist 154 (2002) 275-304.

[49] H. Priwiratama, A. Susanto, Y. Bakhtiar, Effectiveness of mycorrhizae to control basal stem rot disease caused by *Ganoderma boninense* and promoting growth of oil palm seedlings, in: Symposium of Indonesian Mycorrhizae Society, 2011. (in Bahasa)

Journal of Agricultural Science and Technology A 4 (2014) 112-122
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Soil Salinization in Some Irrigated Areas of the Kingdom of Bahrain

Asma Ali Abahussain[1], Abdelhadi Abdelwahab Mohamed[1], Ahmed Ali Salih[1], Ahmad Al Safe[2], Nader Abdul Hamed Mosa[1] and Yahya Othman[1]

1. Desert and Arid Zones Science Program, College of Graduate Studies, Arabian Gulf University, P.O. Box 26671, Manama, Kingdom of Bahrain

2. Ministry of Municipalities Affairs and Urban Planning, Agriculture Affairs, P.O. Box 53, Manama, Kingdom of Bahrain

Received: November 29, 2013 / Published: February 20, 2014.

Abstract: Monitoring and assessment of agricultural land degradation is of vital importance for better land and water management planning and reclamation. It requires setting baseline information and basic analysis at specific time and space. About 33 geo-referenced soil sampling spots were selected in two agricultural production locations in the Kingdom of Bahrain to assess the status and preliminary causes of land degradation. Soil samples were taken from 13 sites in Diraz location while 19 samples were taken from Budayyi location. The samples were taken to 90 cm depth at 30 cm intervals. Standard procedures were followed to determine soil physiochemical properties. In addition, field observations on farm condition, distance from the sea, method of irrigation and irrigation water source were taken. Some of the soil samples were deliberately taken from outside the irrigated basins among trees compared with samples taken from inside the actively growing area for comparison. The results indicated that the salinity level was significantly ($P < 0.001$) higher at the 0-30 cm soil depth compared with 30-60 cm or 60-90 cm depths in both locations. The distance from the sea did not show clear correlation with surface soil salinity in Budayyi area compared with Diraz. Both locations showed significantly higher salinity levels on samples taken outside the actively growing areas compared with those taken from within. The effect is more prominent at the 0-30 cm depth. The observed variability on salinity levels may be attributed to farm management practices and deteriorating quality of ground water. Thus, agricultural land degradation in Bahrain cannot be attributed to ground water deterioration alone. The use of tertiary treated sewage water (TSE) may ease the pressure on ground water, but the pH of the TSE should be carefully monitored and managed with proper studies on leaching requirements to avoid further salinity complications.

Key words: Soil degradation, salinization, Kingdom of Bahrain, geo-referenced soil samples.

1. Introduction

Agricultural land degradation has been one of the greatest threats to food production worldwide. The overwhelming development under the era of oil discovery and fast communication has encouraged intentional/non-intentional degradation of water and land resources [1, 2]. Mismanagement of agricultural lands and/or encroachment of urban areas on agricultural lands are just few signs of bad land management practices. Such bad practices may lead to land degradation. Most academic definitions of land degradation are orbiting about loss of biological and/or economic resilience and adaptive capacity of land systems [3-5]. Land degradation may result from different causes, however, it is estimated that about 550 million hectares of degraded land was attributed to agricultural mismanagement [6]. The same source estimated that of this area 400,000 km^2 were affected by soil salinization and water logging. Salinization was estimated to affect about 20-30 million hectares of the irrigated 260 million hectares [7, 8]. Young [9] stressed the need for continuous soil monitoring at

Corresponding author: Ahmed Ali Salih, professor, research field: soil science. E-mail: ahmedalis@agu.edu.bh.

national levels as global assessment would be unsuccessful without such level of data. One of the most important causes of land degradation is salinization. Salinization of agricultural lands in arid areas is mainly attributed to inappropriate practices regarding crop choice, irrigation method, fertilizer application, bad quality of irrigation water and poor drainage.

Despite the small area of the Kingdom of Bahrain, agriculture was once one of the main sources of income. Idrisi, a famous Arab geographer during the 12th century, described Bahrain as fertile land abundantly producing corn and dates. The total agricultural land during late sixties was estimated to be about 6,000 hectares [10]. Since Bahrain is characterized by arid climate with mean annual winter rainfall of about 72 mm, the natural springs and groundwater were the main source of supplementary irrigation. Two distinct seasons prevail in Bahrain with winter from November to March, temperature ranging between 10 °C and 20 °C while summer extents from April to October with temperature reaching up to 48 °C in June and July. A drastic shift came after the oil discovery and the introduction of drilling wells during the early thirties which later brought an unprecedented social and economical development. Such development has attracted population increase that exerted more pressures on ground water and land resources. For example, arable land decreased by 70%, from 71.40 km^2 in 1998 to 21.60 km^2 in 2008. The area planted with date palm increased from 7.20 km^2 to 12.10 km^2 during the same period, while the area planted with alfalfa remained constant at about 5.60 km^2, the rest of the area is dedicated mostly to vegetable crops [11]. The number of water production wells has increased from one in 1924 to 165 in 1940 and from 325 in 1955 to 1,126 in 2008 [11]. It was not so long when the overexploitation of the limited water resources reflected on ground water deterioration, drying of natural springs and increased soluble salts concentration from 3,400 to 11,000 ppm [12]. Sowar [13] found that the piezometric level of the Damam aquifer over Bahrain has dropped by about 1.5 m during the period 1991-2001 while the water salinity level in some areas of Manama and Muharraq had exceeded 15,000 mg/L. Few studies have addressed soil degradation in Bahrain, e.g., the study conducted in the Karranah village and the Island of Nabih Saleh [14]. The study concluded that ground water salinization, soil texture and mal-farming practices were among the main reasons for soil salinization in the studied areas. Other evidence can be drawn from Ref. [15] which considered man as the dominant impact factor, since the rate of natural geomorphological process is considered to be relatively slow in Bahrain owing to its arid environment. On the other hand, ground water deterioration and the reduction on agricultural areas using remote sensing and GIS technologies have been taken into account in some recent studies [16-19].

Most recently, there is a general direction to expand the utilization of tertiary treated sewage effluent (TSE) for irrigation in Bahrain to ease the pressure on groundwater abstraction.

The main objective of this study is to assess the status of land degradation in the main agricultural area in the Kingdom of Bahrain. The study also aims at understanding the relationship between land degradation and some land management practices such as irrigation methods, farming practices and source of irrigation water while providing baseline information for future studies in some geo-referenced agricultural areas in the Kingdom of Bahrain.

2. Materials and Methods

The two study areas Diraz (2.41 km^2) and Budayyi (1.48 km^2), are the most important production area in the country. More than 33 geo-referenced soil samples were taken from farms in Diraz and Budayyi agricultural production locations (Fig. 1). No systematic grid sampling was possible since the accessibility of

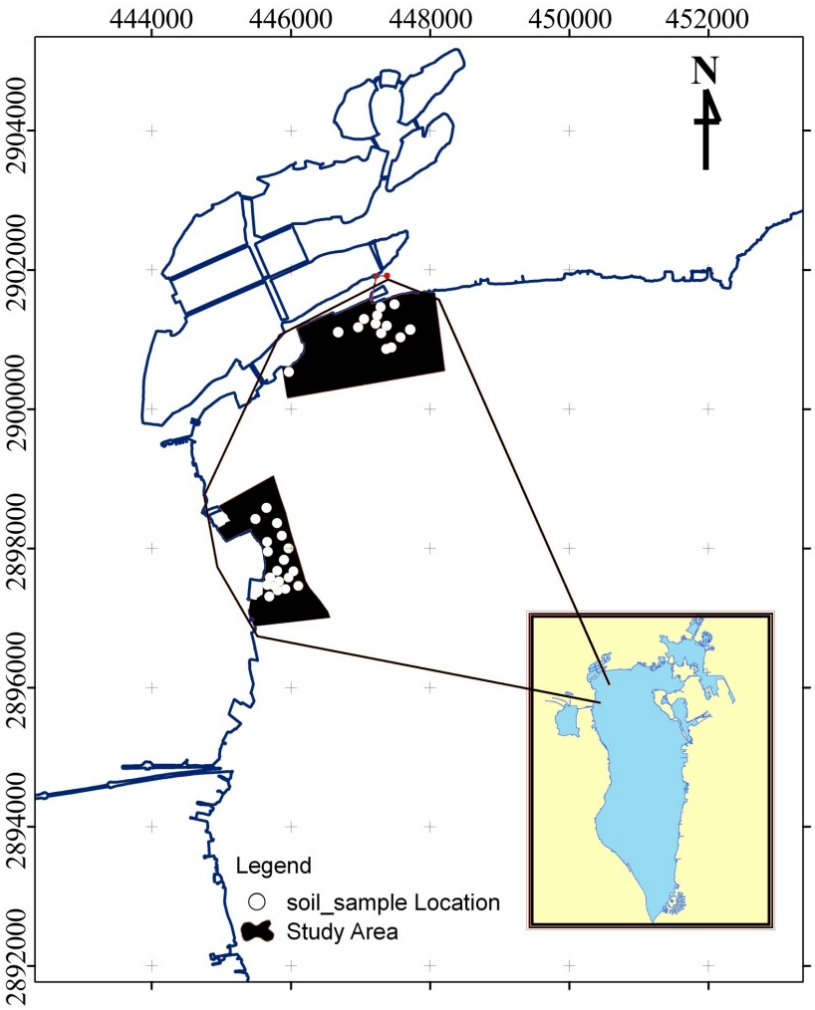


Fig. 1 Location of soil samples shown as white dots.

certain farms was hindered by the availability of residences and the permission for data collection. A soil profile was made within each selected site. The first site represents the agricultural area in the Northern parts at Diraz with 13 completed soil samples to the depth of 90 cm at 30 cm intervals. Those samples were separately analyzed for each specific soil depth. The second location was within the most important agricultural lands with 19 completed sampling spots at Budayyi area. Fig. 1 shows the sampling sites superimposed on Bahrain map. Some of the soil samples were taken from outside the irrigated area to represent uncultivated soil. The collected soil samples were air-dried, crushed, and passed through a 2-mm sieve. Standard procedures were followed to determine soil physiochemical properties [20-23]. The analysis was carried out in the Soil Laboratory of the Arabian Gulf University. Soil texture was determined using the hydrometery method. Soil pH and EC parameters were given from soil saturated water extract using pH meter and conductivity bridge. The amount of soluble and exchangeable Na, K, Ca and Mg were determined using the Flame Photometer. Carbonates and bicarbonates were determined from saturated soil extracts using the titration method with H_2SO_4 N 0.1 to pH 8.3 and 4.5, respectively [24]. Soluble chloride was determined by titration with $AgNO_3$ N 0.01. It is important to mention that the exchangeable sodium percentage (ESP) was calculated using the measured

sodium adsorption ratio (SAR) according to the model presented by Ref. [25]. Direct field observations were also taken during the soil sampling process. These included but were not limited to current farm cropping system, irrigation method and source of irrigation water, presence of crust and/or salt accumulation on surface and ground water level. The distance of the soil sample location from the sea was also considered in the analysis where the GPS was used to measure the shortest distance between the sample location and sea using GIS technique and finally the sampling points with respect to the area under active irrigation activity (inside and outside the irrigation area).

3. Results and Discussion

According to the land capability map [26], the sampled locations are liable to salinization. Preliminary analysis of the EC data revealed high variability within and between the two locations at the 30 cm depth. Table 1 shows some descriptive statistics of EC in the two locations while Figs. 2 and 3 show the spatial and quantitative distribution of salinity levels in the two locations at the three depths increments. Thorough examination of Table 1 in conjunction with Figs. 2 and 3 leads to the following points:

(1) Higher salinity levels were observed at Budayyi area compared with Diraz;

(2) Salinity level decreased with soil depth at both locations;

(3) Within each location the surface salinity levels at 0-30 cm depth were significantly higher than the lower depth increments, while the distance of the sampling point from the sea did not show any specific pattern regarding the salinity at both location;

(4) There is more vertical variability (soil depth) within the same sampling point in Budayyi location than within samples in Diraz location;

(5) Both locations showed higher salinity levels on samples taken just outside the actively growing areas compared with those taken within. The effect is more prominent at the 0-30 cm depth.

Those results suggest other contributing factors such as farm management practices and farm activities.

The spatial distribution of salinity in Diraz using inverse distance smoothing on the sampled locations superimposed on the map (Fig. 4), indicates clear leaching effects of irrigation as indicated by the higher levels of salinities on samples taken outside the growing area (black dots) compared to those taken from inside the plots (white dots). It is worth mentioning that the two extreme values shown in Fig. 4 were also linked with deserted farms.

Similarly, Budayyi location has shown higher levels of salinity especially at the surface on samples taken from outside the growing area (Fig. 5). Those high surface salinities decreased with depth as shown for the 60-90 cm (Fig. 6). Further, extreme data points shown in Figs. 4-6 were attributed to management as field observations in Diraz and Budayyi indicated prolonged in-active farm status that led to salt concentration by evaporation and absence of leaching by irrigation water (Tables 2 and 3).

Table 1 Some descriptive statistics of EC (dS/m) in Diraz and Budayyi locations.

Description	Diraz soil depth (cm)			Budayyi soil depth (cm)		
	0-30	30-60	60-90	0-30	30-60	60-90
Mean	38.5	16.5	13.9	60.8	26.1	17.0
Standard error	12.65	1.98	1.71	11.26	4.66	3.03
Standard deviation	45.62	7.14	6.18	50.35	20.82	13.55
Sample variance	2,081.38	50.98	38.20	2,534.89	433.63	183.69
Kurtosis	1.184	-1.141	0.775	-0.003	2.615	4.598
Skewness	1.517	0.427	0.812	0.743	1.592	2.226
Minimum	5.37	7.51	5.96	4.56	4.19	5.60
Maximum	136.00	28.90	27.90	175.70	86.10	55.80

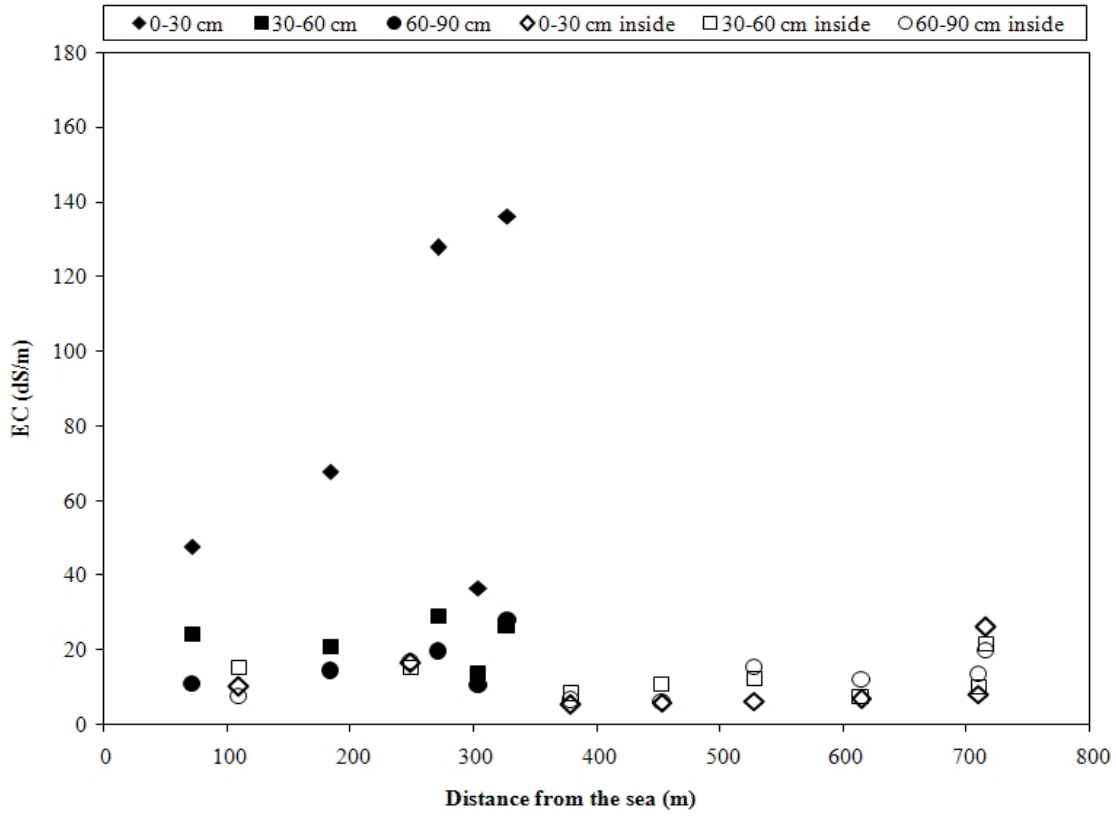


Fig. 2 Spatial and quantitative distribution of salinity in Diraz with filled marks representing samples taken outside the growing area.

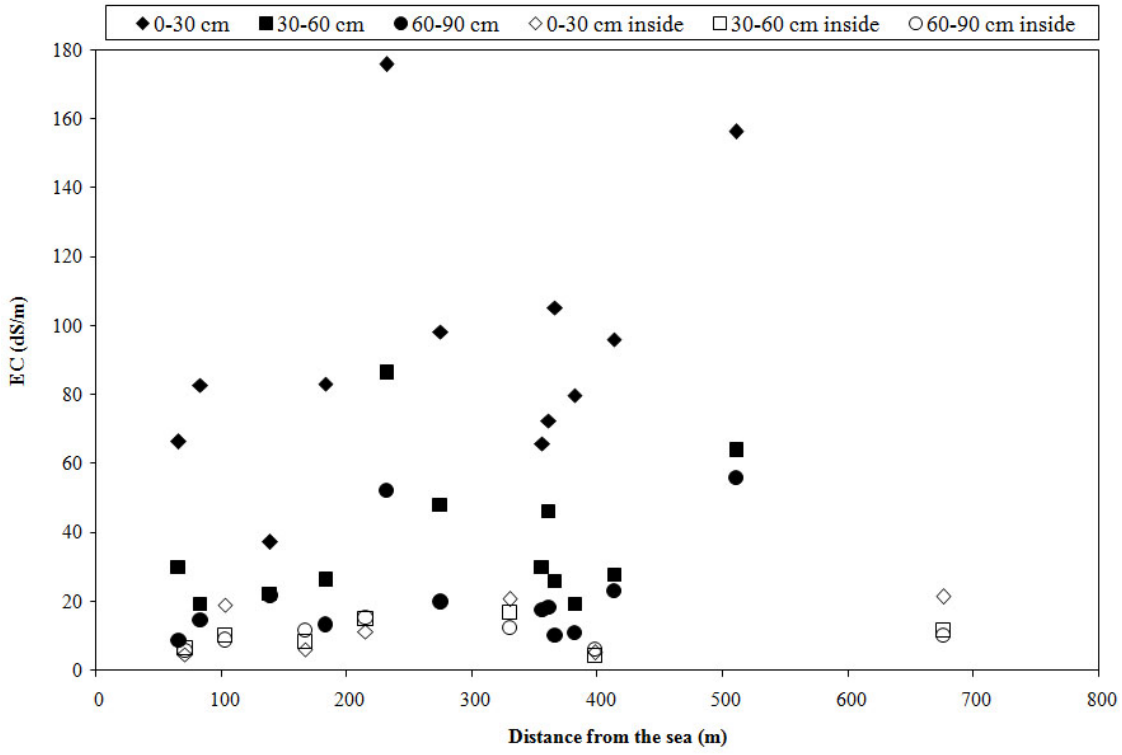


Fig. 3 Spatial and quantitative distribution of salinity levels in Budayyi with filled marks representing samples taken outside the growing area.

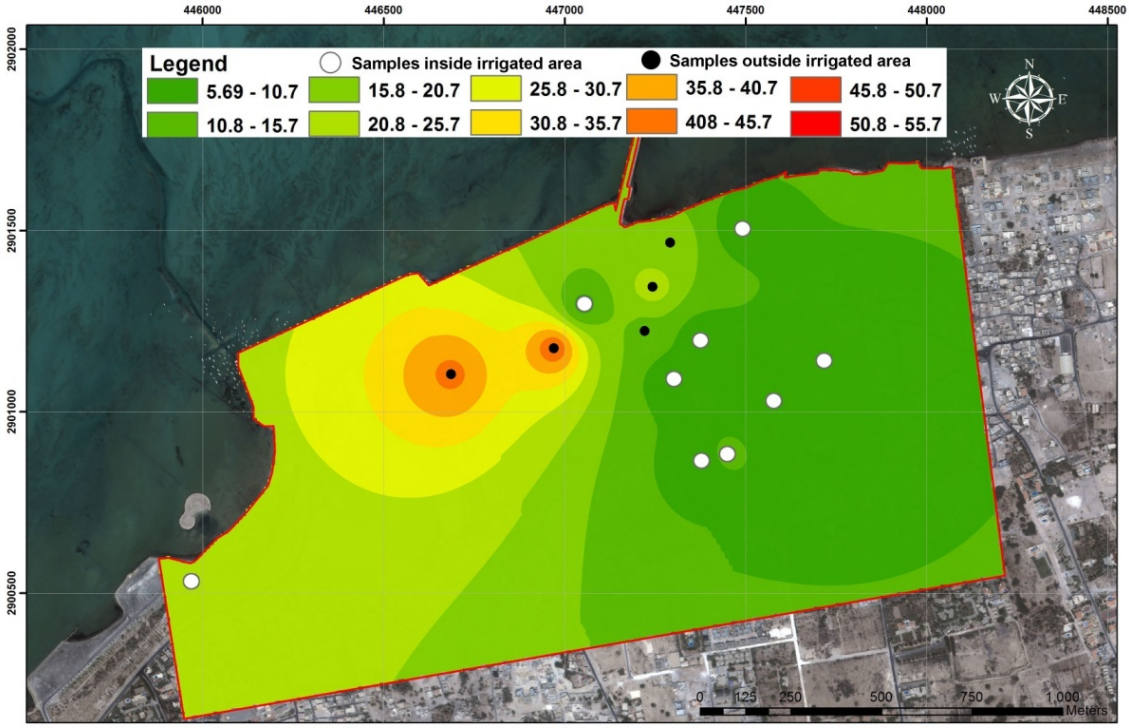


Fig. 4 Distribution of EC (dS/m) at 0-30 cm soil depth in Diraz area.

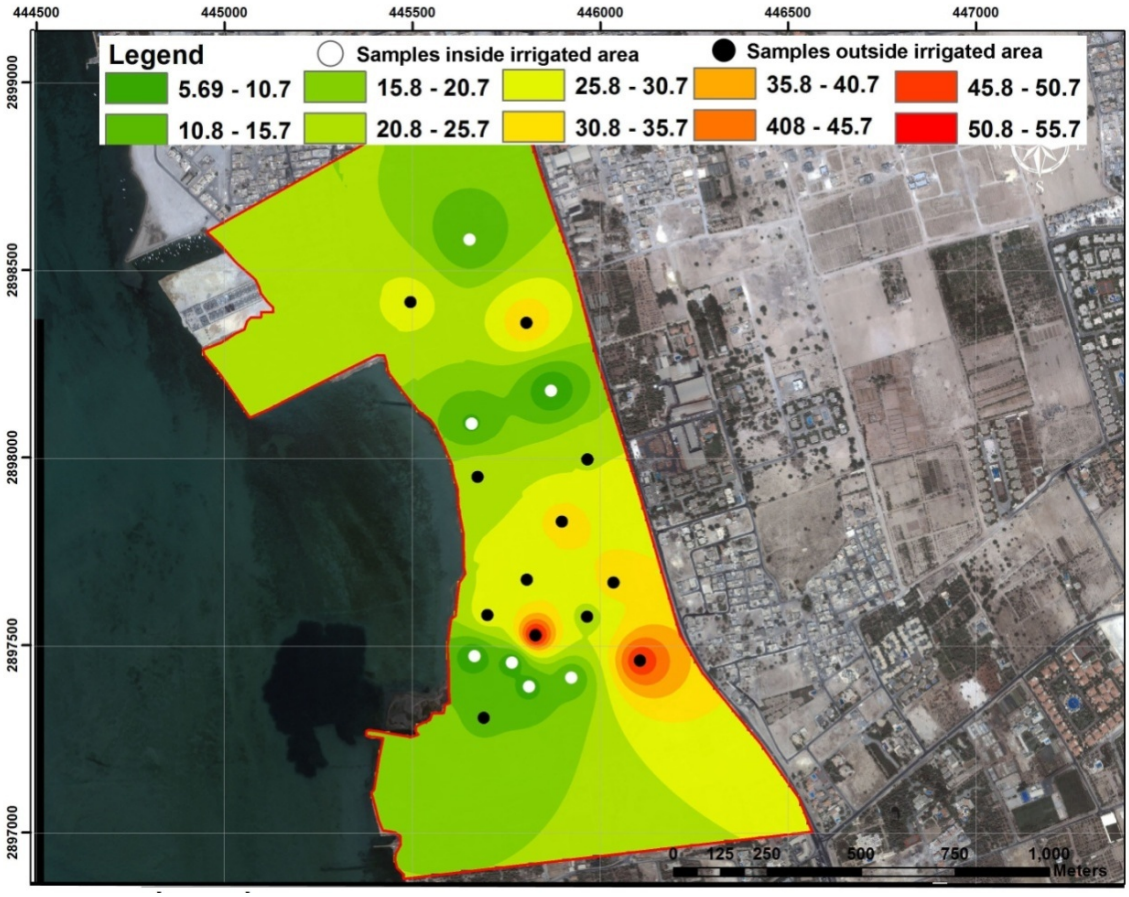


Fig. 5 Distribution of EC (dS/m) at the 0-30 cm soil depth in Budayyi.

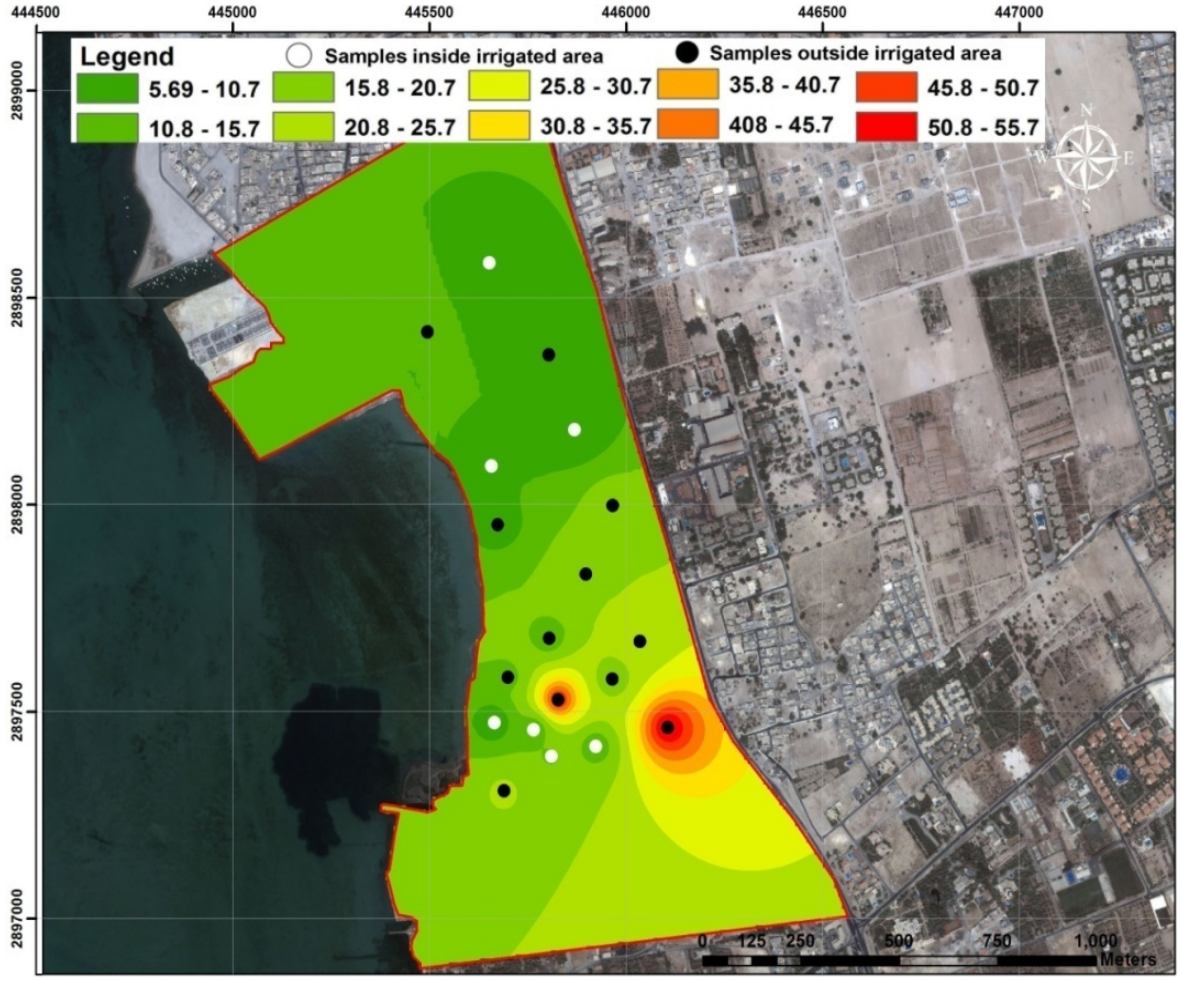


Fig. 6 Distribution of EC (dS/m) at the 60-90 cm soil depth in Budayyi.

Table 2 Some chemical and physical properties measured at 0-90 cm depth arranged according to distance of samples from the sea in Diraz location.

Distance from the sea (m)	Sodium absorption ratio			Exchangeable sodium %			pH	Texture	General farm description	Sample location	Irrigation method	Water source
	Soil sampling depth (cm)											
	0-30	30-60	60-90	0-30	30-60	60-90	0-60	0-90				
72	4.4	7.4	44.8	4.9	8.8	39.3	7.7	Sandy loam	Active farm	Outside basins	Flood	GW
110	5.7	17.7	5.4	6.7	19.9	6.3	7.1	Sandy loam	Active farm	Inside	Flood	GW
184	58.4	16.7	13.3	45.9	19.0	15.5	7.7	Sandy loam	Active farm	Outside basins	Flood	GW
249	10.5	9.0	10.0	12.4	10.7	11.9	6.9	Loamy sand	Active farm	Inside	Flood	GW
272	107.2	16.7	11.1	61.1	19.0	13.2	6.9	Sand	Active farm	Outside basins	NA	NA
304	24.0	13.6	10.7	25.4	15.8	12.6	7.7	Loamy sand	Active farm	Outside basins	NA	NA
328	70.1	16.7	17.9	50.5	19.0	20.1	7.1	Sand	Deserted farm	Barren area	NA	NA
379	5.4	7.0	5.6	6.3	8.3	6.5	7.9	Loamy sand	Active farm	Inside	Flood	GW
453	4.8	8.9	7.4	5.6	10.6	8.8	7.9	Loamy sand	Active farm	Inside	Flood	NA
528	6.5	8.3	11.2	7.7	9.9	13.3	7.8	Loamy sand	Active farm	Inside	Flood	GW
615	5.7	4.4	7.4	6.7	4.9	8.8	8.7	Sandy loam	Active farm	Inside	Flood	GW
710	6.7	6.9	10.2	7.9	8.2	12.1	8.0	Loamy sand	Active farm	Inside	Flood	GW
716	17.3	15.5	13.8	19.6	17.7	16.0	7.9	Loamy sand	Not cultivated	Between basins	NA	NA

NA: not available; GW: groundwater; TSE: tertiary treated sewage effluent.

Table 3 Some chemical and physical properties measured at 0-90 cm depth arranged according to distance of samples from the sea in Budayyi location.

Distance from the sea (m)	SAR			ESP (%)			pH	Texture	General farm description	Sample location	Irrigation method	Water source
	Soil sampling depth (cm)											
	0-30	30-60	60-90	0-30	30-60	60-90	0-60	0-90				
66	55.8	32.7	8.0	44.8	31.9	9.5	7.4	Loamy sand	Trees	Between trees	NA	GW
71	4.4	4.7	4.3	5.0	5.3	4.8	8.5	Sandy loam	Cultivated basins, forage crops	Inside	Flood	NA
83	35.0	11.3	9.6	33.5	13.4	11.4	7.1	Sandy loam	Not cultivated, salt traces	Bare soil	NA	NA
103	12.6	5.5	5.0	14.8	6.4	5.7	7.9	Sand	No crops when sampled	Inside	Flood	GW
139	19.7	21.9	14.9	21.8	23.7	17.1	7.8	Sandy loam	Active farm	Outside basins	Flood	NA
167	9.1	10.7	8.9	10.8	12.7	10.6	7.9	Sandy loam	No crops when sampled	Inside	Flood	NA
183	70.7	21.0	17.4	50.7	22.9	19.6	7.7	Sandy loam	Not cultivated, salt traces	Bare soil	NA	NA
215	13.6	16.3	10.8	15.8	18.6	12.8	7.9	Sandy loam	Alfalfa basins	Inside	Flood	GW
232	139.6	40.8	25.4	67.2	37.1	26.6	7.7	Sandy loam	Trees	Between trees	NA	NA
275	62.9	35.1	13.1	47.8	33.6	15.3	7.3	Sandy loam	Trees	Between trees	NA	NA
330	8.9	7.7	6.4	10.6	9.2	7.5	7.9	Sandy loam	No crops when sampled	Inside	Flood	NA
356	32.0	21.2	13.2	31.5	23.0	15.4	7.6	Sandy loam	Greenhouse	Outside	Drip	GW
361	57.7	41.8	17.7	45.6	37.7	19.9	7.3	Loamy sand	10 years fallow	Bare soil	NA	TSE
366	58.6	15.5	9.0	46.0	17.8	10.7	7.5	Loamy sand	three years fallow, surface crust	Bare soil	Flood	GW
382	47.4	17.8	7.3	40.7	20.0	8.6	7.7	Loamy sand	three years fallow	Bare soil	Flood	GW
398	3.2	4.8	3.7	3.4	5.5	4.1	8.1	Sand	Cultivated basins	Inside	Flood	GW
413	47.0	20.8	14.9	40.5	22.8	17.1	7.5	Sandy loam	Trees, salt on surface	Between trees	NA	NA
511	78.9	29.9	32.3	53.5	30.0	31.7	7.3	Sandy clay loam	15 years fallow, salt on surface	Bare soil	NA	NA
676	17.2	11.0	7.3	19.4	13.0	8.7	7.5	Sand	Greenhouse	Outside	Drip	GW

NA: not available; GW: groundwater; TSE: tertiary treated sewage effluent.

The samples for each depth taken inside the irrigated area were combined from the two locations and analyzed against their corresponding combined samples taken from outside the irrigated area using one factor analysis of variance (ANOVA). Results indicated significant differences regarding EC at all the sampling depth increments as shown in Table 4. The highest differences were observed at the 0-30 cm depth increment with significantly lower EC levels in samples taken within the active irrigated area indicating the importance of leaching.

Table 5 presents the expected leaching fraction for some of the active farms in both locations using Ayers and Westcot relationship of ECe/ECw [27], where ECe represents the average soil salinity at 0-60 cm (dS/m) and ECw is the actual groundwater salinity

Table 4 EC (dS/m) means and statistical analysis for the samples taken from inside and outside of the irrigated area combined for both locations.

Depth (cm)	Samples inside irrigated area	Samples outside irrigated area	Level of significance
0-30	11.5	84.6	$P = 0.001$
30-60	11.5	33.5	$P = 0.001$
60-90	11.1	20.0	$P = 0.05$

Table 5 Calculated leaching fraction (LF) for some active farms based on average soil salinity at 0-60 cm depth and actual groundwater salinity in Diraz and Budayyi locations.

Location	Distance from sea (m)	Average ECe (dS/m)	ECe/ECw	LF
Diraz	110	12.7	2.6	0.07
	249	15.8	3.2	0.05
	379	6.7	1.4	0.20
	453	8.3	1.7	0.14
	528	9.3	1.9	0.11
	615	7.2	1.5	0.18
	710	9.1	1.9	0.12
	716	23.8	4.9	0.02
Budayyi	71	5.5	1.1	0.27
	103	14.4	2.9	0.06
	167	7.1	1.4	0.18
	215	12.9	2.6	0.07
	330	18.6	3.8	0.04
	398	4.6	0.9	0.36
	676	16.3	3.3	0.05

levels which were found to be 4.9 dS/m and 5.0 dS/m for Diraz and Budayyi, respectively. The analysis of $CaCO_3$ content (%) in the two locations indicated clear correlation of $CaCO_3$ content with the distance from sea in Diraz area, especially for the 0-30 cm layer (R^2 = 0.77, Fig. 7a). On the other hand, Budayyi did not show such clear trend (Fig. 7b). This may suggest the need for more research on the pedology of Budayyi location. Further spatial analysis of $CaCO_3$ using inverse distance and ArcGIS technique showed that the gradient of $CaCO_3$ concentration is rather taking a south-north direction especially for the 0-60 cm soil layer (not shown here).

Examination of SAR in conjunction with EC has shown that higher SAR coincides with higher EC (Fig. 8). High SAR has the potential to impair soil structure and thus soil permeability; it is particularly true when the EC of irrigation water or soil water is not high enough to counteract the negative effect of sodium on soil structure.

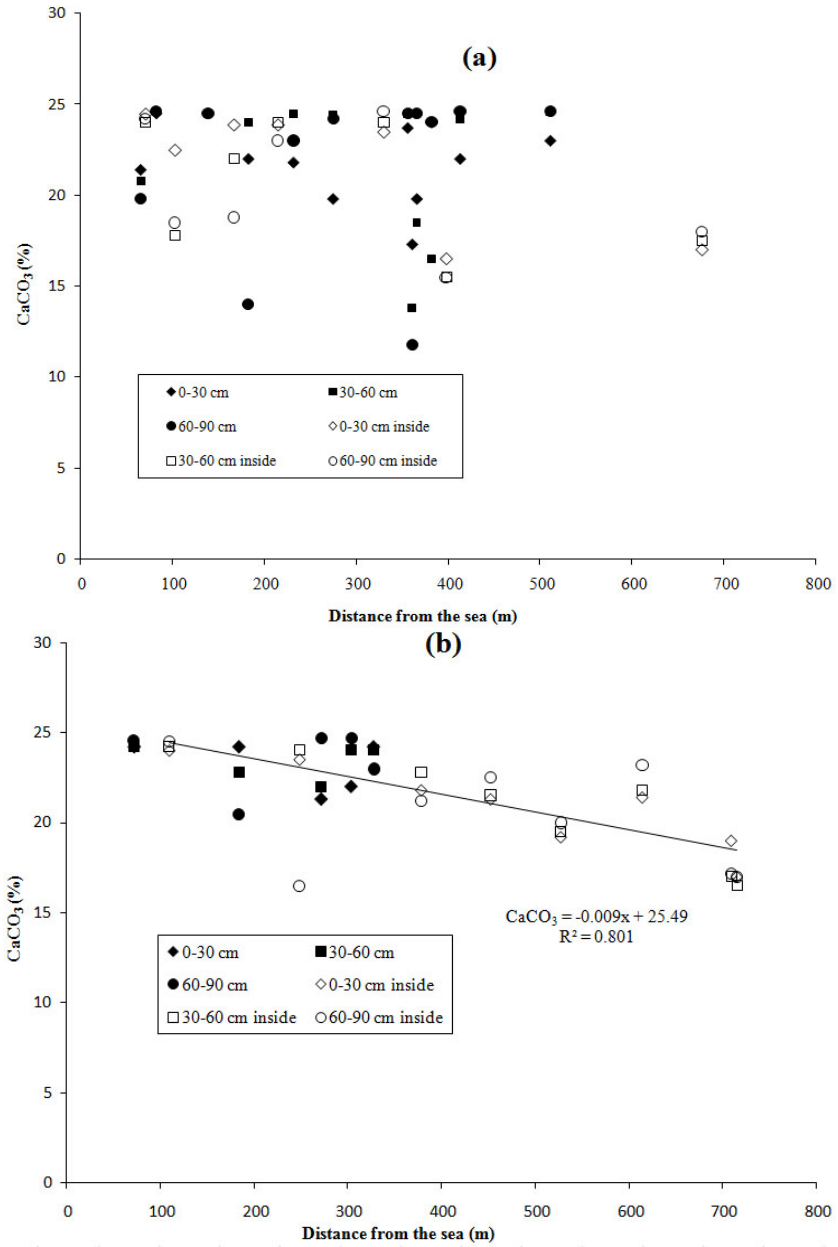


Fig. 7 Spatial and quantitative distribution of $CaCO_3$ (%) in Budayyi (a) and Diraz (b) showing soil depth, samples locations and correlation.

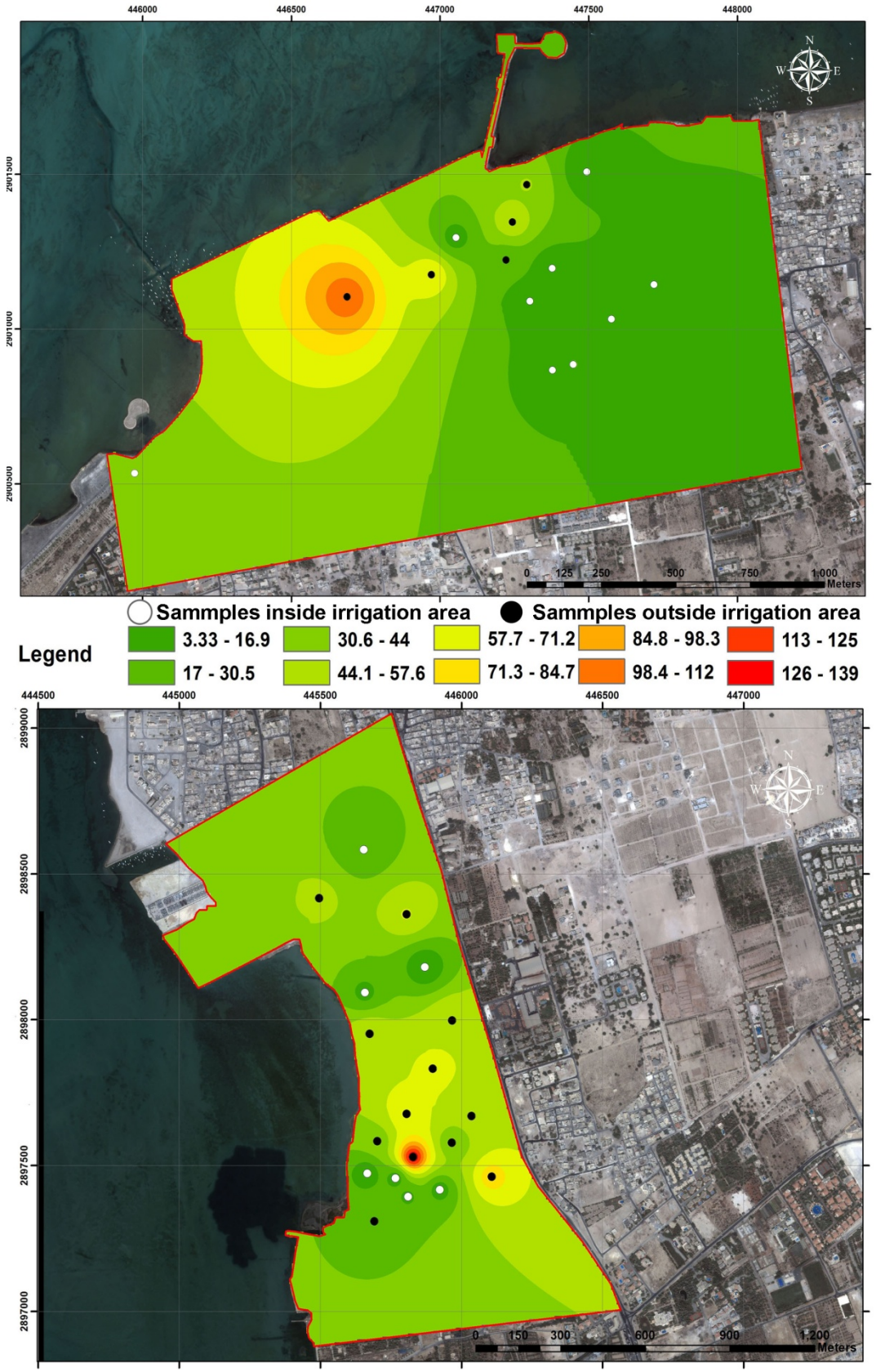


Fig. 8 Visual and quantitative distribution of SAR at 0-30 cm depth in Diraz (above) and Budayyi (below).

4. Conclusions

Salinity is the main cause of land degradation in Bahrain owing to worsening ground water quality and poor land management practices. Leaching requirements should be designed according to the prevailing salinity levels in soils and irrigation water to enhance sustainable agricultural production. More use of TSE will reduce the pressure on groundwater and reduce fertilizer use since TSE contains some of the essential nutrients. A baseline was set and geo-referenced for further detailed studies and follow-up regarding agricultural land degradation in Bahrain. However, further research is needed to investigate the quality and safety of using TSE for irrigation in addition to the effects of irrigation systems on salinity.

References

[1] G. Gardner, Worldwatch paper # 131—Shrinking fields: Cropland loss in a world of eight billion, 1996.

[2] D. Hinrichsen, Winning the Food Race, Baltimore, Maryland, 21202-4012, USA: Population Information Program, Center for Communication Programs, 1997.

[3] W. Dean, M. Hoffman, M. Meadows, S. Milton, Desertification in the semiarid Karoo, South Africa: Review and reassessment, Journal of Arid Environments 30 (1995) 247-264.

[4] C. Holing, Understanding the complexity of economic, ecological and social systems, Ecosystems 4 (2001) 390-405.

[5] Intergovernmental Panel on Climate Change (IPCC), Climate Change 2001: The Scientific Basis, J.D. Houghton (Ed.), Cambridge University Press, Cambridge UK, 2001.

[6] Food and Agriculture Organization (FAO), Our Land Our Future, FAO and United Nations Environment Program, 1996.

[7] K. Tanji, N. Kielen, Agricultural drainage water management in arid and semi-arid areas, Irrigation and Drainage Paper 61, FAO, Rome, 2001.

[8] F. Ghassemi, A. Jakeman, H. Nix, Salinization of land and water resources: Human causes, extent, management and case studies, Canberra, Australia, 1995.

[9] A. Young, Soil monitoring: A basic task for soil survey organizations, Soil Use and Management 7 (3) (1991) 126-129.

[10] Italconsult, Water and Agricultural Studies in Bahrain, FAO, Min. Agriculture Water, Rome, Italy, 1971.

[11] M.A. Al-Noaimi, Water Statistics up to 2006, Ministry of Municipalities Affairs and Agriculture, Agriculture Affairs, 2011.

[12] NAPCD, The National Action Program to Combat Desertification in Bahrain, Agricultural Affairs, Ministry of Works and Agriculture, State of Bahrain, 2007.

[13] M. Sowar, Ground water quantity and quality assessment of the Damam aquifer in the Kingdom of Bahrain, 1991-2001, M.Sc. Thesis, Desert and Arid Zones Sciences Program, Arabian Gulf University, Kingdom of Bahrain, 2002.

[14] Z.J. Al-Herz, Agricultural land degradation by salinity in the Kingdom of Bahrain: Case study for Karranah village and Nabih Saleh Island, M.Sc. Thesis, Arabian Gulf University, 2006.

[15] J. Doornkamp, D. Brunsden, D.K.C. Jones, Geology, geomorphology and pedology of Bahrain, Published by Geo Abstracts Ltd., University of East Anglia, Norwich, UK, 1980.

[16] W. Zubari, Spatial and temporal trends in groundwater resources in Bahrain, 1992-2002, Emirates Journal for Engineering Research 10 (1) (2005) 57-67.

[17] M.M. Abudarahim, Development of three-dimensional numerical model for the management of the Damam aquifer in the Kingdom of Bahrain, M.Sc. Thesis, Arabian Gulf University, 2010.

[18] M.K. Al Meraikhi, Determining and analyzing sustainable development density using the urban carrying capacity in the Kingdom of Bahrain, 1998-2009, M.Sc. Thesis, Arabian Gulf University, 2011.

[19] Bahrain's Second National Communication, Kingdom of Bahrain, Public Commission for the Protection of Marine Resources, Environment and Wildlife, 2012.

[20] L. Alison, L. Bernstein, C. Bower, J. Brown, M. Fireman, G. Hatcher, Diagnosis and improvement of saline alkali soils, Agriculture Handbook, No. 60, United States Salinity Laboratory, 1954.

[21] S. Thien, J. Graveel, Laboratory Manual for Soil Science, Agriculture and Environmental Principles, S. edition, McGraw-Hill Companies, Inc., United States of America, 1997.

[22] C.A. Black, Methods of Soil Analyses, Part 1 and Part 2, Transportation Research Board, Washington DC, National Academy of Science, 2004.

[23] J. Ryan, G. Estefan, A. Rashid, Soil and Plant Analysis Laboratory Manual, 172 (Ar), 2003.

[24] L. Richard, Diagnosis and improvement of saline and alkali soils, USDA Agricultural Handbook, No. 60, Washington DC, United States Department of Agriculture, USA, 1954.

[25] W.C. Robbins, Coefficients for estimating SAR from soil pH and EC data and calculating pH from SAR and EC in salinity models, Arid Soil Research and Rehabilitation 7 (1984) 29-38.

[26] M.S. Ali, The Soil of Bahrain (Ar), Manama, Kingdom of Bahrain: Ministry of Works and Agriculture, Agricultural Research Administration, 1999.

[27] R.S. Ayers, D.W. Westcot, Water quality for agriculture, FAO Irrigation and Drainage Paper 29, Food and Agriculture Organization of the United Nations, Rome, 1985, p. 174.

Journal of Agricultural Science and Technology A 4 (2014) 123-128
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Effect of Enriched Cattle Manure on Soil Nutrient Status, Nitrogen Uptake and Yield of Tea (*Camellia sinensis*)

Vivian Moroamoche Kekana[1], Isaiah Tabu[1], David Kamau[2] and Robert Obura[1]

1. Department of Crops, Horticulture and Soils, Egerton University, Egerton 20115, Kenya

2. Department of Chemistry, Tea Research Foundation of Kenya, Kericho 20200, Kenya

Received: March 6, 2013 / Published: February 20, 2014.

Abstract: Inorganic fertilizer NPK (nitrogen, phosphorus and potassium) (S) 25:5:5:5 is generally recommended for optimum yield and quality of tea (*Camellia sinensis*). Non-judicious use of this inorganic fertilizer however acidifies the soils and pollutes the environment. Integrated soil fertility management (ISFM) which involves the combined use of organic and inorganic fertilizer is recommended for improved crop yield and soil health. An experiment was carried out to determine the effect of enriching cattle manure with different ratios of inorganic fertilizers (OM: NPKS at ratios 1:2 and 1:4), and rates on soil nutrient status, nitrogen uptake and yield of tea in the east of Rift Valley, Kenya. Enriching manures and organic manure up to a rate of 150 kg N/ha increased the level of P mature leaf. A higher N and K level in the mature leaf was observed when NPKS was applied at higher rates. In the soil, fertilizer rate up to 150 kg N/ha showed higher pH and K where organic manure and enriched manures were applied while NPKS treatment showed higher P content throughout the soil depths. Enriching organic manures with inorganic fertilizers increased yield significantly.

Key words: Cattle manure, enrichment, nitrogen, tea (*Camellia sinensis*), yield, inorganic fertilizer, ISFM.

1. Introduction

Tea (*Camellia sinensis*), one of the leading cash crops in Kenya can remain in production for up to 100 years. The regular harvesting of the crop (two leaves and a bud) however implies that nutrients are continuously mined from the soil. Nitrogen is one of the most important nutrients for tea production. A yield of 4,000 kg made tea/ha removes about 160-200 kg N, 12-15 kg P_2O_5 and 84-100 kg K_2O from the soil [1]. Replacement of the lost nutrients is crucial for sustainable production of the crop.

In Kenya, a fertilizer rate of 100-200 kg N/ha in form of compound fertilizer (NPKS) is recommended for optimal yield and quality of tea. Many farmers use more than the recommended rates in a bid to realize high crop yield [2]. The high fertilizer rates are not only expensive, but they could also acidify the rhizosphere and pollute the water masses [3]. Cattle manure, one of the alternative nutrient sources especially in acid soils is however limited by quality and quantity and is laborious [4, 5]. Integrated soil fertility management (ISFM) narrowly defined as the combined use of organic and inorganic fertilizers is recommended because of the synergistic effects that result in high crop yield and improved chemical, physical and biological soil conditions [6, 7]. The use of ISFM has mainly involved enrichment before application or physical combination during application especially in annual crops. The benefits of ISFM have been demonstrated in the annual crop systems, but the challenge is how much, in what proportions and when the different fertilizer types are to be used. An experiment was carried out to determine the effect of fertilizer types and rates on soil properties, nitrogen uptake and yield of tea.

Corresponding author: Isaiah Tabu, associate professor, research fields: integrated soil fertility and crop management systems. E-mail: immtabu@yahoo.com.

2. Materials and Methods

2.1 Site Description

A field experiment was established in the year 2000 at Kangaita, latitude, 0°26′ S, longitude 37°15′ E and altitude of 2,020 m above sea level on the slopes of Mt Kenya at East of Rift Valley, Kenya. The soils are red clay classified as Humicacrisols [8].

2.2 Treatment Combination

A high yielding clone TRFK 31/8 was used. The treatments were: 1, NPKS 25:5:5:5; 2, cattle manure; 3, enriched fertilizer of cattle manure: NPKS 25:5:5:5 at a ratio of 2:1; 4, enriched fertilizer of cattle manure: NPKS 25:5:5:5 at a ratio of 4:1.

Each of the treatments was applied at equivalent rates of 0, 75, 150 and 225 kg N/ha/year. Cattle manure was sourced from the nearby farmers' fields and standardized using the N content. The fertilizers were applied during the first week of September 2010 and 2011.

2.3 Experimental Design

The trial was a four by four factorial experiment laid out in a randomized complete block design (RCBD) and replicated three times. Each plot was (10.98 × 5.46) m^2. The net plot comprising of (7 × 14) bushes spaced at (1.22 × 0.61) m^2. The whole trial was surrounded by a complete guard-row of tea bushes.

2.4 Laboratory Analysis

2.4.1 Soil Analysis

Six soil samples from each plot were taken from top (0-20 cm) and sub soil (40-60 cm) during the dry season using a soil auger. The soil was air dried, sieved (2 mm) and analyzed for its pH, P, K and cation exchange capacity (CEC) [9]. Cattle manure was also analyzed for its chemical composition [9].

2.4.2 Nitrogen Uptake

Leaf analysis has been used for a long time as a diagnostic tool in many perennial crops. Fifty mature leaves/net plot were sampled and taken to the laboratory for oven drying for 24 h at 105 °C and milled using the coffee miller (®Ramtons). Nitrogen was determined using Kjeldahl method, P by spectrophotometer and K by the flame photometer [9].

2.5 Yield

Tea was plucked at 7-10 d interval and the weight/plot was recorded at every plucking round. The yields were converted to kg made tea per hectare per year (kg mt/ha/year) using the following equation:

$$Y = (n \times a \times 0.225)/b$$

where, Y is kg made tea/ha, n is green leaf yield per plot, a is plant population per hectare, 0.225 is the factor converting green leaf to made tea [10] and b is the number of plants per plot.

2.6 Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using SAS version 9.0 statistical software package. Means were separated by Student-Newman-Keuls (SNK). Soil data with high coefficient of variations (CV) was transformed using $\log_e (x+1)$.

3. Results and Discussion

3.1 Soil Properties

The manure and soil chemical characteristics are shown in Table 1 below. The soils are quite acidic and low in nutrient level.

Soil pH was significantly affected by the fertilizer type (Table 2). Cattle manure had the highest soil pH.

Table 1 Chemical characteristics of the soil and manure.

Parameter	Soil	Manure
pH (H_2O) 1:1	3.6 ± 0.1	7.3
P (mg/kg)	17.8 ± 2.6	0.28
K (mg/kg)	149.0 ± 0.4	1.31
Ca (mg/kg)	155.5 ± 0.3	0.89
Mg (mg/kg)	19.0 ± 0.4	0.24
C	nd	22.8
C:N ratio	nd	22

± standard error; nd: not determined.

Table 2 Effect of fertilizer type and rate on soil pH, P and K.

Parameter	Fertilizer type	Rate (kg N/ha/year)				Mean
		0	75	150	225	
Soil pH	T1 (NPKS)	3.56[a]	3.20[b]	3.32[b]	3.32[b]	3.35[b]
	T2 (OM)	3.57[a]	3.64[a]	3.81[a]	3.84[a]	3.72[a]
	T3 (T2:T1) 1:2	3.57[a]	3.33[b]	3.24[b]	3.25[b]	3.34[b]
	T4 (T2:T1) 1:4	3.57[a]	3.35[b]	3.26[b]	3.26[b]	3.36[b]
	Mean	3.57	3.38	3.40	3.42	
P	T1 (NPKS)	16.67[a]	19.33[a]	34.00[a]	44.00[a]	28.50[b]
	T2 (OM)	16.67[a]	17.33[b]	22.67[b]	21.67[b]	20.34[a]
	T3 (T2:T1) 1:2	16.67[a]	17.33[b]	22.67[b]	23.67[b]	19.09[a]
	T4 (T2:T1) 1:4	16.67[a]	19.33[a]	24.33[b]	24.33[b]	20.92[a]
	Mean	16.67	18.33	25.92	28.42	
K	T1 (NPKS)	5.00[a]	4.90[a]	4.97[b]	5.17[ab]	5.01
	T2 (OM)	5.03[a]	4.94[a]	5.89[a]	5.90[a]	5.44
	T3 (T2:1) 1:2	5.00[a]	4.94[a]	4.64[b]	4.60[b]	4.80
	T4 (T2:T1) 1:4	4.99[a]	4.90[a]	4.69[b]	4.65[b]	4.80
	Mean	5.01	4.92	4.95	4.86	

Means with different letters within the same block are significantly different.

The increase in soil pH with organic manure was due to the fact that during decomposition of organic materials, level of available cations was increased [11]. In addition, organic acid anions may have complexed Al^{3+} ions that contribute to acidity. This was confirmed from the reduced level of exchangeable acidity by cattle manure (Table 3). Oxidation of organic acid anions is generally considered as the main contributor to the increased soil pH [12]. Enriched manures and NPKS reduced soil pH probably because of the higher amount of NH_4^+ and NO_3^- which underwent mineralization and nitrification, and releasing H^+ ions in the soil [13]. Increased soil pH with addition of organic manure and reverse with use of inorganic fertilizers and enriched manures has been noted in central highlands of Kenya [14].

Generally, soil pH decreases with increase in fertilizer rate except for organic manure where it increases with increase in fertilizer rate. The decrease in soil pH with increase in N rate applied in tea soils has previously been reported [8]. The effect is probably because of the increased level of NH_4^+. The soil pH decreased with increase in soil depth where NPKS fertilizer was applied.

This is consistent with the principle that inorganic N fertilizers increase soil acidification not only on the top soil but also in the subsoil [15-17].

The NPKS and enriched manures led to high P in the soil probably because of differences in nutrient levels. Phosphorus level also significantly increased with the increase in fertilizer rate. Similar results have been reported on the effects of rates and frequency of applications on soil chemical properties [15].

The soil extractable K was significantly affected by the fertilizer type and rate (Table 2). Cattle manure had the highest available K^+ followed by NPKS and lastly enriched manures. The results were expected because cattle manure supplies K^+ on mineralization and has the acid humus that helps in adsorption of K^+ leading to reduced leaching [18]. Large quantities of NH_4^+-N ions in enriched manure may have however displaced K^+ from the soil matrix. When NPKS was used compared to the control, lower soil K^+ has previously been noted [16].

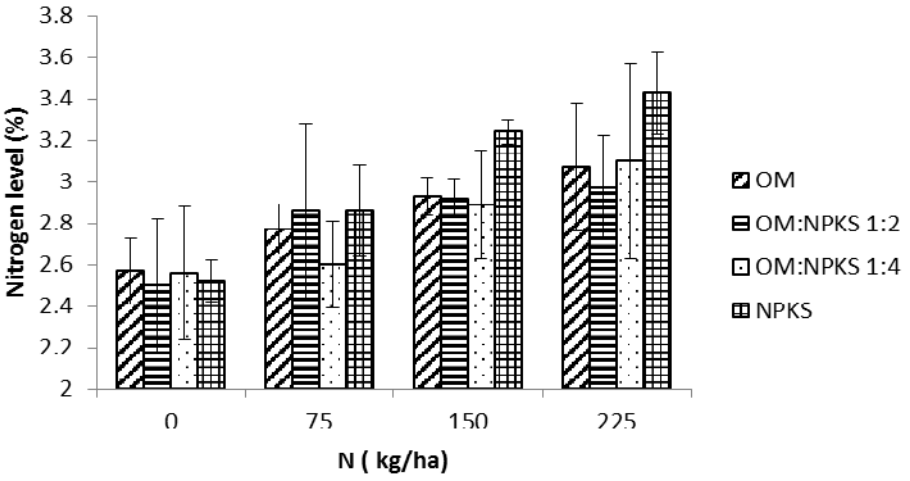
3.2 Nitrogen Uptake

The N content in the mature leaf did not differ significantly with fertilizer type for both seasons (Fig. 1).

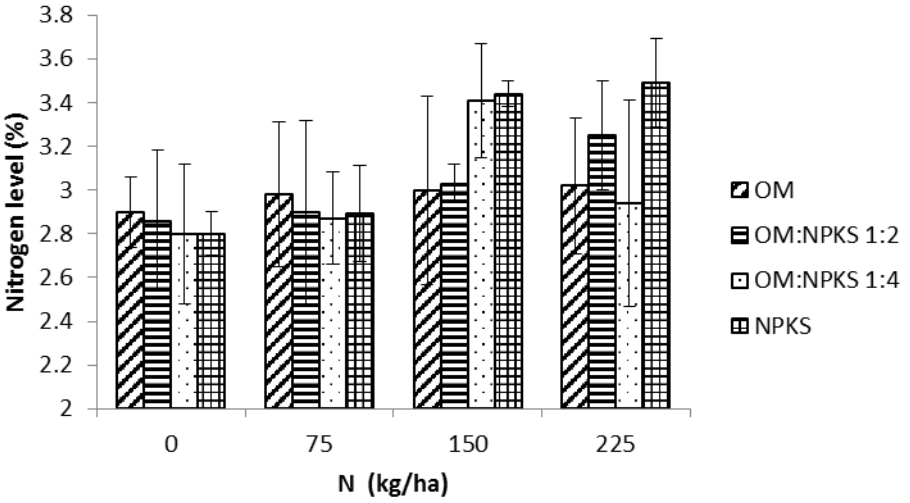
Table 3 Effects of enrichment and rate on exchangeable acidity in the soil at Kangaita.

Fertilizer type	Exchangeable acidity				Al^{3+} concentration			
	Rate N (kg/ha)				Rate N (kg/ha)			
	0	75	150	225	0	75	150	225
Cattle manure (OM)	0.09[a*]	0.07[a]	0.05[a]	0.04[a]	0.71[a]	0.07[a]	0.08[a]	0.37[a]
OM: NPKS 1:2	0.09[a]	0.07[a]	1.00[b]	0.38[b]	0.71[a]	0.73[b]	1.02[b]	0.71[b]
OM: NPKS 1:4	0.09[a]	0.07[a]	1.00[b]	0.39[b]	0.71[a]	0.73[b]	1.03[b]	1.03[c]
NPKS	0.09[a]	0.07[a]	1.00[b]	1.01[c]	0.71[a]	0.72[b]	1.03[b]	1.35[d]

*Means with different letters within the same block are significantly different.



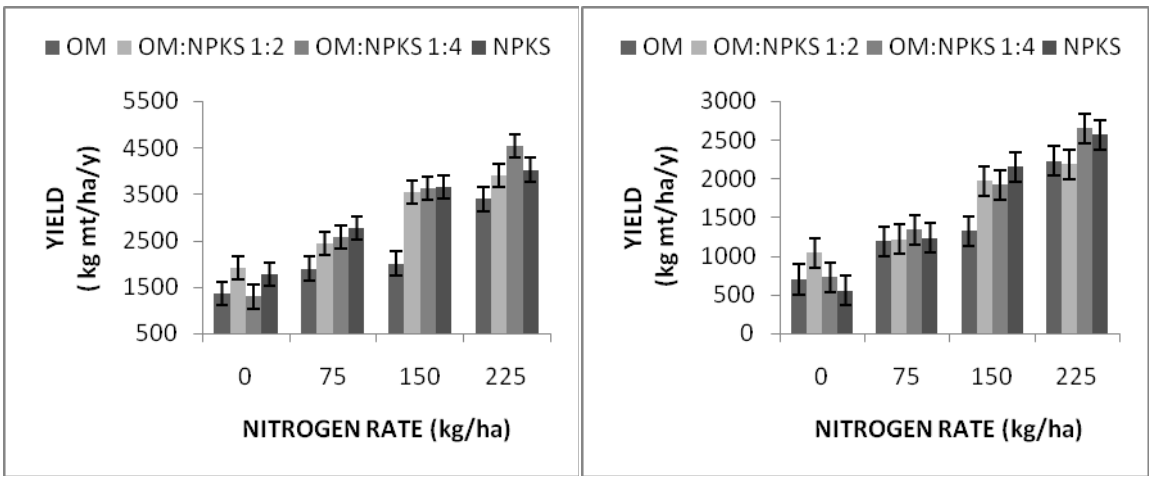
(a) Season 1



(b) Season 2

Fig. 1 Effects of fertilizer type and rate on the N content of the mature leaf at different seasons.

Thus NPKS fertilizer, enriched cattle manure or sole cattle manure can be used to maintain tea bush. Using either 25:5:5:5 or 20:10:10 did not result in different N contents in the mature leaf [19]. Fertilizer rate significantly increased the N content in the mature leaf in the first season. The seasonal variation might be due to severe drought which was observed between February and March, 2011. An increase in N content



(a)Year 2010 (b) Year 2011

Fig. 2 Effects of fertilizer type and rate on annual crop yield at different years.

in the mature leaf with increase in fertilizer rate has also been observed [19]. Increasing fertilizer rates under organic manure resulted in lower N content compared to NPKS and enriched manures. Lower N content in the mature leaf may be due to slow release of nutrients [4]. These results suggest that organic manure alone cannot supply adequate N for sustainable tea productivity.

3.3 Crop Yield

The fertilizer types were applied based on N level, the most important nutrient enhancing yield. Yield varied significantly with fertilizer type and rate (Fig. 2).

The annual crop yield variation is a common characteristic in tea where several factors including temperatures, rainfall, amount of rainfall and distribution vary [20]. In 2011 lower rainfall was received which resulted in lower yields compared to year 2010. Enriched manures had the highest yield followed by NPKS and organic manure especially at higher fertilizer rates. The response of tea yield to the increase in N fertilizer rate has been reported in several studies [16, 17]. The increase in yield with enriched manures is consistent with the principle that ISFM which affects both the soil biological and physical properties that results in improved nutrient retention and nutrient release patterns thus increasing yield [7]. The low nutrient status coupled with the slow release of nutrients from organic manure maybe responsible for the low yields [21]. It shows that organic manure or NPKS alone cannot sustain tea production.

4. Conclusions

Use of organic manure resulted in higher soil pH, P and K in both mature leaf and in the soil. Although enriched manures showed lower K in the mature leaf and lower P and K in the soil, the use of enriched manures resulted in higher soil pH, higher crop yield and P in the mature leaf compared to the use of standard tea fertilizer (NPKS). Enriched manures have shown to reduce P fixation in the soil resulting in higher uptake by the crop hence lower amount remaining in the soil. Enriched manures can thus be used instead of the inorganic fertilizer (NPKS) to enhance tea production through integrated soil fertility management.

Acknowledgments

The authors are grateful to the Tea Research Foundation of Kenya (TRFK) for funding the research project and providing the site for field experiments.

References

[1] Tea Research Foundation of Kenya, Tea Research Foundation of Kenya Strategic Plan (2010-2015), 2010, p. 4.

[2] P.O. Owuor, W.K. Ng'etich, M. Obanda, Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard, Journal of the Science of Food and Agriculture 80 (2000) 439-446.

[3] S.C. Killpack, D. Buchholz, Nitrogen in the Environment, Department of Agriculture and Extension, University of Missouri, 1993.

[4] R. Gupta, R. Kumar, S.K. Tripathi, Study on agroclimatic condition and productivity pattern of sugarcane in India, Sugar Tech 6 (3) (2004) 142-149.

[5] J.K. Lekasi, J.C. Tanner, S.K. Kimani, P.J.C. Haarris, Managing Manure to Sustain Smallholder Livelihoods in the East African Highlands, HDRA Publications, 2001, p. 32.

[6] M.M. Mishra, K.K. Kapoor, K. Chander, R.D. Laura, Sustainable agriculture: The role of intergrated nutrient management—A review, International Journal of Tropical Agriculture IX (3) (1991) 153-173.

[7] B. Vanlauwe, J. Wendt, J. Diels, Combined application of organic matter and fertilizer, in: G. Tian, F. Ishida, J.D.H. Keating (Eds.), Sustaining Soil Fertility in West Africa, SSA Special Publication, No. 58, Soil Science Society of America, Madison, USA, 2001.

[8] S.J. Kebeney, D.M. Kamau, C.O. Othieno, W.K. Ng'etich, P.O. Owuor, Changes in soil chemical properties and leaf nutrients content in tea due to nitrogen fertilizer rates and application intervals, Tea 31 (1) (2010) 22-27.

[9] J.R. Okalebo, K.W. Gathua, P.L. Woomer, Laboratory Methods of Soil and Plant Analysis: A Working Manual, 2nd ed., TSBF-CIAT and SACRED Africa, Nairobi, Kenya, 2002.

[10] The Tea Research Foundation of Kenya, Tea Growers Handbook, 5th ed., 2002.

[11] R.J. Haynes, M.S. Mokolobate, Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: A critical review of the phenomenon and the mechanisms involved, Nutrient Cycling in Agroecosystems 59 (2001) 47-63.

[12] A.D. Noble, I. Zenneck, P.J. Randall, Leaf litter ash alkalinity and neutralization of soil acidity, South African Journal of Plant Soil 179 (1996) 293-302.

[13] S. Garrison, The Chemistry of Soil, 2nd ed., Oxford University Press, New York, 1990.

[14] C.D. Shisanya, M.W. Mucheru, D.N. Mugendi, J.B. Kung'u, Effect of organic and inorganic nutrient sources on soil mineral nitrogen and maize yields in central highlands of Kenya, Soil Tillage Research 103 (2009) 239-246.

[15] Y.W. Dogo, P.O. Owuor, J.K. Wanyoko, C.O. Othieno, High rates of nitrogen on tea at high altitudes, VII: The effects of rates and frequency of applications on soil chemical properties in Mount Kenya area, Tea 15 (1) (1994) 17-26.

[16] D.M. Kamau, J.K. Wanyoko, K.W. Nyabundi, K. Sitienei, Effects of organic manures on tea yields and soil properties in Kenya, in: Tea Organic-Low Carbon International Symposium, Guangyuan, China, 2011, pp. 14-28.

[17] J.K. Wanyoko, Advances in fertilizer use in Kenyan tea industry, Tea 18 (2) (1997) 123-131.

[18] B.C. Barbora, Fertilizer use in tea, Two and Bud. 38 (1991) 1-2.

[19] D.M. Kamau, P.O. Owuor, J.K. Wanyoko, Long term effects of rates and types of nitrogenous fertilizers on high yielding clone AHP S15/10 tea (I): Yields and nutrients uptake, Tea 24 (1) (2003) 14-20.

[20] P.O. Owour, C.O. Othieno, D.M. Kamau, J.K. Wanyoko, W.K. Ng'etich, Effects of long term fertilizer use on high yielding tea clone AHPS15/10: Yields, International Tea Journal 7 (2008) 19-31.

[21] I. Phukan, A. Baruah, R.M. Bhagat, Influence of combined use of organic, inorganic and bio fertilizer sources on chemical, physical and biological properties of tea soil, Two and a Bud. 55 (2008) 28-35.

Journal of Agricultural Science and Technology A 4 (2014) 129-134
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Ethylene Stimulation of Rubberwood (*Hevea brasiliensis*) Increases the Water Permeability of Lumber

Banyat Cherdchim and Rossarin Sudchada

Faculty of Sciences and Industrial Technology, Prince of Songkla University, Surat Thani Campus, Muang District, Surat Thani 84000, Thailand

Received: November 14, 2013 / Published: February 20, 2014.

Abstract: *Hevea brasiliensis* Muell. Arg. is an important industrial crop for natural rubber production. Latex biosynthesis occurs in the cytoplasm of highly specialized latex cells and latex bleeds out when the bark is tapped. Ethylene stimulation acts by increasing latex flow to the cells of inner bark from the latex cells, increasing yield and may affect the physical properties of rubberwood. The aim of this work was to assess the permeability properties of ethylene treated rubberwood (TRW) relative to untreated rubberwood (URW), because in wood industrial technology, permeability relates to bondability and wood preservative treatments. The *Hevea* samples were of PRIM 600 strain, from 20-25 years old rubber trees. The TRW rubber trees had been stimulated by ethylene gas for six years. The rubberwood specimens were collected at a single plot of plantation to minimize variations in soil fertility, environmental exposures and silvicultural treatments at Tumbon Chaibury, Amphor Chaibury, Suratthani Province, Thailand. The moisture contents (MC) of fresh rubberwood were significantly different ($P < 0.05$) at 75% for TRW and 64% for URW. The permeability experiment followed Darcy's law, and the hydrostatic pressure was controlled. The average 0.005 Darcy water permeability of TRW was significantly higher than the 0.001 Darcy for URW. Water absorptions during 4 h water immersion of rubberwood blocks differed significantly, and TRW had higher absorption than URW also across 6 d of immersion. Scanning electron microscope (SEM) imaging showed anatomical effects that contribute to the fivefold permeability increase.

Key words: Ethylene, permeability, rubberwood, water absorbance, water diffusion.

1. Introduction

Currently rubber tree farmers stimulate rubber trees with ethylene to improve the latex yield of trees 15 years or older, and the trees are felled around 25-30 years age. While ethylene stimulation benefits latex yield, it might also impact the physical properties of rubberwood lumber. When the stimulation used holes bored into wood, it generated injures and changed the color of lumber decreasing the value of lumber. These problems have decreased with a new generation of ethylene treatments such as RRIMFLOW technique, where stimulating ethylene passes through the wood bark without drilled holes. However, the physical, mechanical or other key properties of wood lumber may be affected by ethylene treatment, and this is an area with current gaps in knowledge.

At the Suratthani Rubber Research Center (Thailand) [1], they have studied the moisture content and shrinkage-swelling of ethylene treated rubberwood, but no significant treatment effects were observed. Effects on chemical composition have been studied, and there are reports that ethylene treated rubberwood (TRW) had higher lignin and extractive contents than untreated rubberwood (URW) [2]. Moreover, the TRW had higher resistance to white rot fungi *Schizophyllum commune* and *Garnoderma* spp. than URW, in experiments with 12 weeks of incubation at 22 °C, RH 65%. The penetration rate of boron compounds (disodium octaborate tetrahydrate (DOT)) into TRW was higher than into URW [3]. DOT is a conventional wood preservative chemical

Coresponding author: Banyat Cherdchim, Ph.D., research field: wood technology. E-mail: banyat.c@psu.ac.th.

that induces slight color changes in wood. It is low cost and locally available. The reports have shown increased penetration rate of urea formaldehyde (UF) and bondability for TRW relative to URW [4]. These results suggest that the permeability of TRW is higher than that of URW, but the water permeability has not been determined. Knowing the treatment effects on this fundamental physical property will benefit industrial use of rubberwood enabling informed decisions. While there have been many studies on the latex yield from rubber tree plantations [5-8], the treatment effects on lumber properties have been less well studied. In the current study, we focused on water permeability.

Hevea brasiliensis Muell. Arg. wood is especially attractive because it is a fast growing plantation tree with a high potential for sustainable lumber production, and also otherwise of interest to the wood products industries. The current practice of ethylene stimulating *H. brasiliensis* needs to be assessed for its impacts on wood anatomy, which in turn might affect chemical preservative treatments or adhesive bonding. In this work, Darcy's law [9] was applied to water permeability in wood. The authors also determined the water absorbance and water diffusion rates in wood.

The current research was motivated by the needs of both rubber farmers and the industries that use rubber wood. If the effects of farming practices on wood quality are well understood and can be adapted to, it could increase the value of the wood raw material, what should benefit also the seller. The scope of this research is of necessity quite limited in relation to this general motivation, but relates strongly to the preservation treatment that is of primary importance for wood products.

2. Materials and Methods

2.1 Moisture Content in Wood

The fresh *H. brasiliensis* wood specimens were collected at Tumbon Chaibury, Amphor Chaibury, Suratthani Province, Thailand. The six rubberwood blocks of 30 mm (longitudinal) × 30 mm (tangential) × 30 mm (radial) were oven dried at 103 ± 2 °C for 24 h. The moisture content (MC) of wood was calculated as:

$$MC = \frac{M_G - M_{OD}}{M_{OD}} \times 100\%$$

where, M_G is the green mass of the wood, M_{OD} is its oven-dry mass.

2.2 Permeability of Wood to Water

Six *H. brasiliensis* wood blocks of 150 mm (longitudinal) × 10 mm (tangential) × 5 mm (radial) were oven dried at 103 ± 2 °C for 24 h, then mounted onto the ends of about 2 m long rubber tubes (9 mm in diameter) with about 5 mm length in the longitudinal direction within the tubes. In order to prevent solvent to leak out from the other surfaces of the wood blocks, the rest of the wood blocks were sealed with parafilm (Laboratory Film, Chicago, USA) leaving only the surface of the cross-section at the open ends of wood blocks free. The rubber tubes were fitted onto outlets affixed close to the bottom of a 500 mL plastic container. The plastic container was positioned on a shelf at about 2 m height, and the wood sticks in the rubber tubes were hanging in the opening of a 600 mL bottle standing on the floor. Water was filled into the 500 mL plastic box and with a natural hydrostatic pressure of about 20 kPa (calculated from $P = \rho hg$; P = hydrostatic pressure (Pa), ρ = fluid density (kg/m^3), h = the height (m), g = gravitational acceleration (m/s^2)). The water amount collected in the bottle over 30 min was weighed to determine the permeability of the wood sample. The distilled water was at ambient temperature (30 °C), and in the calculations it was assumed to have 1 cP viscosity. The permeabilities determined are for the axial direction, parallel to the wood fibers.

2.3 Water Absorbance and Water Diffusion in Wood

The wood blocks were cut into four smaller blocks of size 30 mm (longitudinal) × 10 mm (tangential) × 5 mm (radial) each, discarding 1.5 cm from both ends.

These blocks were dried and used in water absorbance determinations. Dried UTR wood blocks (size 30 mm (longitudinal) × 10 mm (tangential) × 5 mm (radial)) served as control. Six wood blocks for each type of wood were immersed into distilled water at ambient temperature, and the mass weighed every 15 min up to 4 h of total immersion time.

In another set of water absorbance experiments, six oven dried blocks of each type of *H. brasiliensis* (30 mm (longitudinal) × 10 mm (tangential) × 5 mm (radial), dried at 103 ± 2 °C for 24 h) were immersed into distilled water at ambient temperature, and mass weighted every day up to 6 d of immersion.

2.4 Statistical Analysis

Group values for all parameters in the tests were compared by Kruskal-Wallis tests (SPSS 8.0 for Windows, USA). In particular the treated vs. control contrast was analyzed.

3. Results and Discussion

The fresh wood moisture contents were determined as 64% for URW and 75% for TRW (Fig. 1), respectively.

During oven drying at 103 ± 2 °C for 24 h, TRW shrunk more than URW, especially in the radial direction (Fig. 2). It suggests that ethylene treatment may impact severely wood shrinkage or moisture induced swelling, and limit applications where dimensional stability is critical.

H. brasiliensis wood blocks of 150 mm (longitudinal) × 10 mm (tangential) × 5 mm (radial) were used to determine the axial permeability and potential treatment effects on it (see "Materials and Methods" for details). The statistically significant treatment effects on permeability to water are illustrated in Fig. 3. TRW had almost five fold permeability relative to URW.

There are reports on permeability (Table 1) of different softwoods and hardwoods from the outer heartwood part [9, 10]. Our wood samples were selected from a part between sapwood and heartwood. The values shown in Table 1 corroborate our permeability values as reasonable for wood samples.

3.1 Effects of Water Immersion on H. brasiliensis Wood

H. brasiliensis wood was tested at room temperature (30 °C). The total mass of each sample was weighed every 15 min to monitor the rate of water absorption. Absorption continued over the observed about 4 h (Fig. 4). After immersion for 1 h, the moisture content (MC) of URW was 21% and 24% for TRW. Throughout the rest of the observation span TRW had about 5% higher MC than URW (Fig. 4). The MC of wood is composed of both bound water and free capillary or pore water [11], but these cannot be distinguished from the time profiles of absorption. The rapid initial water uptake takes place because the sample surfaces were initially dry. Further water absorption into the inner sample must diffuse through that expanding wetted surface layer, and this increasing diffusion distance slows down the absorption rate.

A similar immersion test with distilled water at room temperature (30 °C) was extended to total duration

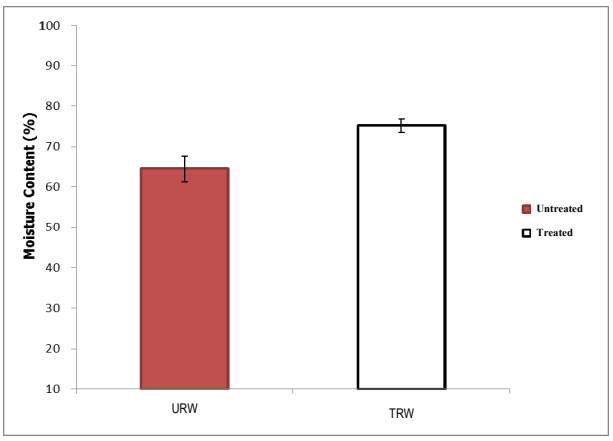
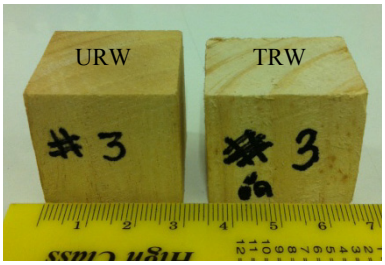
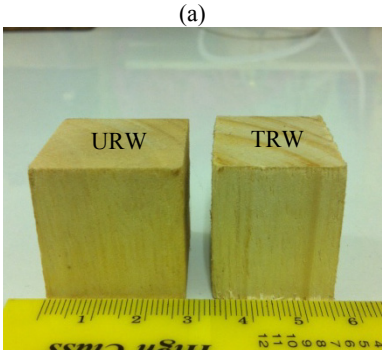


Fig. 1 Moisture contents of *H. brasiliensis* wood blocks of 30 mm (longitudinal) × 30 mm (tangential) × 30 mm (radial) were determined by oven drying at 103 ± 2 °C for 24 h or until stable mass.

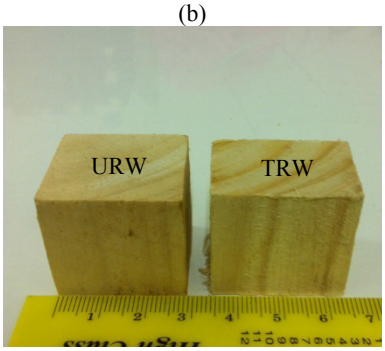
Ethylene treated rubberwood is labeled TRW and untreated rubberwood URW. The averages of six replicates were significantly ($P < 0.05$) different between the treatments based on Kruskal-Wallis analysis.



(a)



(b)



(c)

Fig. 2 Images illustrating the shrinkage of *H. brasiliensis* wood blocks initially 30 mm (longitudinal) × 30 mm (tangential) × 30 mm (radial) cubes, induced by drying at 103 ± 2 °C for 24 h or until stable mass.

Ethylene treated rubberwood is labeled TRW and untreated rubberwood URW. (a) longitudinal wood shrinkage; (b) radial wood shrinkage; (c) tangential wood shrinkage.

of 6 d with daily observations as shown in Fig. 5. TRW samples consistently had higher MC than URW samples at equal immersion time as before. The MC increased consistently throughout the experiments, and the highest MC was about 90%. Factors or mechanisms that contribute to the slow long-term absorption may include the dissolution of gases into water from the pore space, enabling higher water saturation in blind pores and slow swelling of the samples that increases their pore volume.

From prior research [12] we know that radial trunk growth is related to access to water and carbon. Carbon

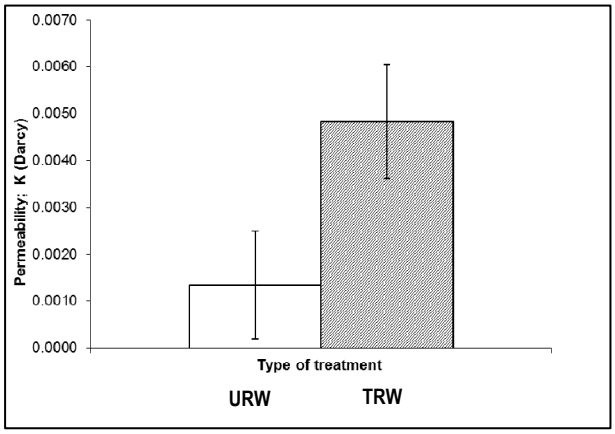


Fig. 3 Permeabilities, K (Darcy) of *H. brasiliensis* oven dried wood blocks when water was forced axially through them over a 30 min period.

Ethylene treated rubberwood is labeled TRW and untreated rubberwood URW, with six replicates in each treatment group the permeabilities differed significantly ($P < 0.05$) between treatments according to Kruskal-Wallis analysis.

Table 1 Water permeability of outer heartwood [9, 10].

Permeability by species	Permeability, Darcy	
	Mean	Standard error
Douglas fir	0.006	0.001
Lodgepole pine	0.001	0.0007
Engelmann spruce	0.007	0.0006
Eucalyptus grandis	0.0042	0.0025

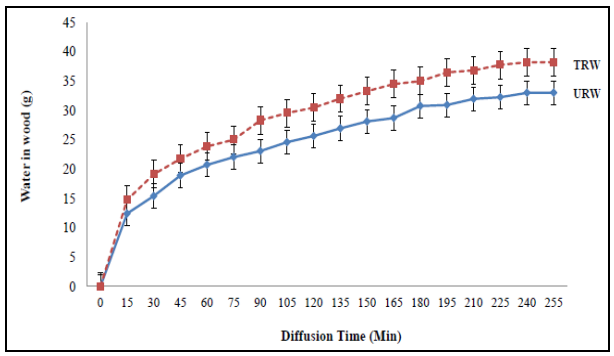


Fig. 4 Water absorption time profiles of immersed *H. brasiliensis* wood blocks of 30 mm (longitudinal) × 15 mm (tangential) × 10 mm (radial), initially oven dry.

Ethylene treated rubberwood is labeled TRW and untreated rubberwood URW with six replicates in each treatment group. The absorption profiles were significantly different.

is the basic chemical element for structural compounds and for storage of metabolic energy, and so are the atoms of water molecules. Stem radial variations reflect four main factors: (1) irreversible radial growth; reversible shrinkage and swelling affected by (2) hydration, (3) thermal expansion and

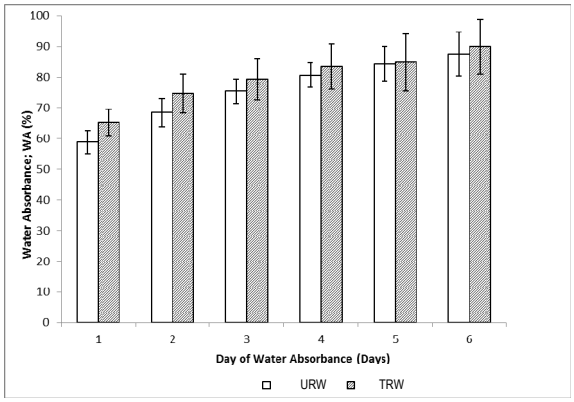


Fig. 5 MC profiles of treated and untreated wood samples which were initially oven dried during immersion in water over 6 d.

(4) contraction and expansion of conducting elements, also responding to internal mechanical stresses [8, 12]. No significant treatment effect on radial trunk growth has been detected [7, 8]. Also high frequency tapping may affect the MC and growth rate of a rubber tree. It has been reported that URW had girth increment superior to TRW [13]. The TRW cases may have suffered from overexploitation and overstimulation. The increased latex yield of TRW necessitates higher consumption of water and carbon sources than by URW, relating to our measurement results (Fig. 1, Table 2). An elemental analysis of C, H, N and O was carried out with a CHNS-O analyzer, CE Instrument Flash EA 112 Series, Thermo Quest, Italy. The analyzer used dynamic flash combustion with left furnace temperature of 900 °C, oven temperature 65 °C, carrier flow 130 mL/min, reference flow 100 mL/min and oxygen flow 250 mL/min. In case of oxygen analysis, the parameters were: right furnace 1,060 °C, carrier flow 130 mL/min and reference flow 100 mL/min. The TRW cases had higher fraction of carbon than the URW (Table 2).

The results presented in Figs. 3-5 indicate that ethylene stimulation enhances permeability and water absorption of rubberwood probably through an effect on porosity. Porosity is defined as the fractional volume of voids that could be filled by a fluid in the solid structure. Ethylene treatment of *Populus alba* L. hardwood causes the cambium to produce more

Table 2 Elemental analysis by % mass of *H. brasiliensis* wood samples from two treatment groups.

Wood	Element (SD) (%)				
	N	C	H	S	O
TRW	0.41 (9.49×10^{-3})	45.09* (0.28)	6.22 (3.47×10^{-2})	< 0.01	41.29 (0.18)
URW	0.40 (5.74×10^{-3})	44.86* (5.28×10^{-2})	6.22 (3.57×10^{-2})	< 0.01	41.22 (0.23)

Ethylene treated rubberwood is labeled TRW and untreated rubberwood URW;
*indicates the element content is significantly different between treatments.

parenchyma, shorter fibers and shorter vessel elements than in control [14]. Application of ethylene to stem of *P. alba* causes abnormal growth and stem anatomy with an increase in wood and bark. The treatment has pleiotropic effects on dimensions of xylem cells and tissue pattern. Moreover, the tangential width of radial wood parenchyma cells increases; the wood cells are big and plasma rich with simple pits of different sizes and shapes. Ethylene treated wood may have normal xylem aside from the increased size of xylem rays that have more and larger cells than rays of the control [14, 15].

The fresh wood densities were determined as 0.94 g/cm^3 for URW and 0.91 g/cm^3 for TRW. The wood pit density and vessel diameter increments are likely causes of increased permeability in ethylene stimulated rubberwood (Fig. 6). The influence of ethylene stimulation on rubberwood structure was imaged with scanning electron microscope (SEM). Figs. 6a and 6b show that the treatment increased vessel diameters (single pore (SP)) to an average of 230-270 μm from 200-240 μm. In the double pores (DP), TRW had vessel diameters of 300-440 μm while the control had 240-380 μm (Figs. 6a and 6b). The simple pits in Fig. 6c had higher density in TRW than in URW (Fig. 6d) increasing permeability (Figs. 3-5). Thus, increment of vessel diameter and wood pit density of TRW are observed factors evidently contributing to water permeability and absorption increases.

4. Conclusions

Ethylene stimulation of rubberwood improved the axial water permeability of lumber relative to rubberwood

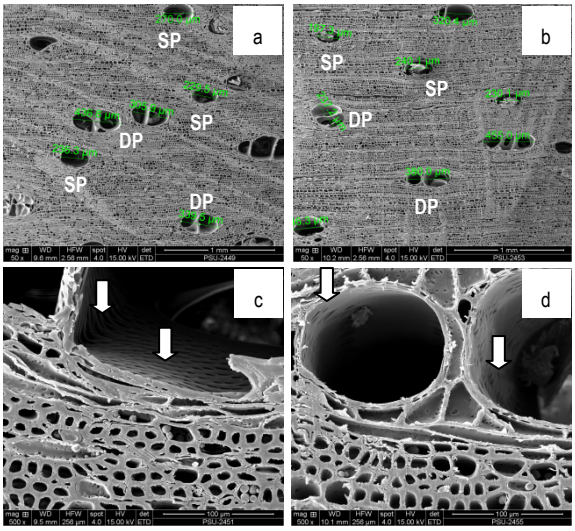


Fig. 6 SEM images of the structure of *H. brasiliensis* illustrate treatment effects contributing to increased permeability.

(a) cross section of TRW, (b) cross section of URW, the pit density is evident in (c) cross section of TRW and (d) cross section of URW; a-b scale bar = 1 mm, b-c scale bar = 100 μm; arrows point to the pits in vessel cell walls.

without ethylene treatment. The increase in axial permeability to water by ethylene treatment of *H. brasiliensis* was about five fold. Scanning electron microscope imaging suggested that ethylene treatment increased the areal number density of pits and the average vessel diameter. These physiological effects likely contributed to increases in water absorption rate and permeability to water of wood.

Acknowledgments

The authors thank Prince of Songkla University for financial support as well as its Scientific Equipment Center. The helpful comments and suggestions from Dr. Seppo Karrila are gratefully acknowledged.

References

[1] K. Sangsing, P. Parechana, W. Prommee, The comparison of wood product, wood quality and properties of ethylene stimulated wood, Journal of Rubber: Suratthani Rubber Research Center (Thailand) 1 (2009) 10-15. (in Thai)

[2] T. Damkleang, T. Poonchauy, B. Cherdchim, Study of effect of tapping by using ethylene gas on the chemical properties and decay resistance of rubberwood by termites and fungi, Student Project, Prince of Songkla University, Thailand, 2011.

[3] S. Yaowalerd, W. Yingprasert, The investigation of bondability of rubberwood tapped by using ethylene gas, Student Project, Prince of Songkla University, Thailand, 2013.

[4] S. Khongao, A. Phetarwut, The penetration rate of boron compounds in ethylene stimulated rubberwood, Student Project, Prince of Songkla University, Thailand, 2013.

[5] A. Dusotoit-Coucaud, P. Kongsawadworakul, L. Maurousset, U. Viboonjun, N. Brunel, V. Pujade-Renaud, et al., Ethylene stimulation of latex yield depends on the expression of a sucrose transporter (*HbSUT1B*) in rubber tree (*Hevea brasiliensis*), Tree Physiology 30 (2010) 1586-1598.

[6] R. Lacote, O. Gabla, J.M. Eschbach, F. Rivano, K. Dian, E. Gohet, Long-term effect of ethylene stimulation on the yield of rubber trees is linked to latex cell biochemistry, Field Crops Research 115 (2010) 94-98.

[7] T. Sainoi, S. Sdoodee, The impact of ethylene gas application on young-tapping rubber, Journal of Agricultural Technology 8 (4) (2012) 1497-1507.

[8] U. Silpi, P. Thaler, P. Kasemsap, A. Lacointe, A. Chantuma, B. Adam, et al., Effect of tapping activity on the dynamic of radial growth of *Hevea brasiliensis* trees, Tree Physiology 26 (2006) 1579-1587.

[9] D.C. Markstrom, A. Hann, Seasonal variation in wood permeability and stemoisture content of three mountain softwoods, Forest Service U.S. Department of Agriculture, 1972.

[10] M.R. Silva, G.O. Machado, J. Deiner, C.C. Junior, Permeability measurements of Brazilian *Eucalyptus*, Material Research 13 (3) (2010) 281-286.

[11] B. Cherdchim, N. Matan, B. Kyokong, Effect of temperature on thermal softening of black sweet-bamboo culms (*Dendrocalamus asper* Backer) in linseed oil, Songklanakarin Journal of Science Technology 26 (6) (2004) 855-866.

[12] F. Daudet, T. Améglio, H. Cochard, O. Archilla, A. Lacointe, Experimental analysis of the role of water and carbon in tree stem diameter variations, Tree Physiology 30 (2004) 1586-1598.

[13] M.S. Traore, M. Diarrassouba, K.M. Okoma, K.E. Dick, E.F. Soumahin, L.F. Coulibaly, et al., Long-term effect of different annual frequencies of ethylene stimulation on rubber productivity of clone GT1 of *Hevea brasiliensis* (Muell. Arg.) in South East of Cote d'Ivoire, Agricultural and Biology Journal of North America 2 (2011) 1251-1260.

[14] U. Junghans, R. Langenfeld-Heyser, A. Polle, T. Teimann, Effect of auxin transport inhibitors and ethylene on the wood anatomy of Poplar, Plant Biology 6 (2004) 22-29.

[15] C.H.A. Little, L. Eklund, Ethylene in relation to compression wood formation in *Abies balsamea* shoots, Trees 13 (1999) 173-177.

Journal of Agricultural Science and Technology A 4 (2014) 135-140
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Gillnet Selectivity and Length at Maturity of Nile Tilapia (*Oreochromis niloticus* L.) in a Tropical Reservoir (Amerti: Ethiopia)

Mathewos Hailu
Ziway Fisheries Resources Research Center, Ziway 229, Ethiopia

Received: November 20, 2013 / Published: February 20, 2014.

Abstract: The selectivity of gillnets for *Oreochromis niloticus* in Amerti reservoir (9°63′ N, 37°23′ E) was determined from gillnets with four mesh sizes (60, 80, 100 and 120 mm). Four selectivity models (a normal model assuming fixed spread, a normal model assuming that spread is proportional to mesh size, a lognormal model and a gamma model) were fitted to the data by using the share each length's catch total (SELECT) method. A total of 657 specimens of *Oreochromis niloticus* were caught (12.0-35.5 cm total length, T_L). The sizes at first sexual maturity were 21.5 cm T_L and 18.9 cm T_L, respectively, for male and female *Oreochromis niloticus*. The lognormal selectivity curve provided the best fit to the data according to model deviance estimates with optimum selectivity of 16.66, 22.26, 27.78 and 33.38 cm T_L for the 60, 80, 100 and 120 mm mesh sizes, respectively.

Key words: Gillnet, mesh selectivity, Nile tilapia, SELECT.

1. Introduction

Oreochromis niloticus (Nile tilapia) is native fish species of East African lakes [1]. The species has been introduced to many parts of the world and has adapted to wide range of environmental variables [2]. Adaptation of Nile tilapia to various environments is due to its ability to both protracted spawning period and variability in length at first maturity in response to environmental and fisheries related stress [3-5].

Nile tilapia is one of the most important commercial fish species in Ethiopia [6]. Regardless of the adaptability and tolerance to various environmental and anthropogenic impacts, high demand for Nile tilapia in Ethiopia has led to the decline of fish stocks in some Ethiopian lakes and reservoirs [7]. The decline of *Oreochromis niloticus* is aggravated due to lack of regulations on fishing effort, minimum landing size and minimum mesh size of gillnets.

Managing fisheries requires a combination of limits on area, time, gear, size, species and effort [6]. Fishing gears greatly influence the size frequency of targeted species [8, 9], and understanding the reproductive biology and selectivity of the fishing gears in a particular environment is important for managing a targeted species.

Due to the size-selective nature of gillnets which are widely used by fisheries that target *Oreochromis niloticus*, mesh size regulations can be an effective tool for managing the size composition of catches [10]. The share each length's catch total (SELECT) model is considered to be the most robust indirect method to estimate gear selectivity [10, 11]. The method is widely used to obtain selectivity of various fishing gears [12].

Recently (February, 2013), regional proclamation has been ratified on fish resource conservation, food

Corresponding author: Mathewos Hailu, assistant researcher, research field: aquatic ecology. E-mail: lemathewos@gmail.com.

safety and aquaculture. This document puts considerable emphasis on regulation, permits and the role of the fishery inspector. Thus, this study aimed at identifying size at first maturity and selectivity of gillnets for Nile tilapia.

2. Materials and Methods

2.1 Sampling

The study was conducted in Amerti reservoir (9°63′ N, 37°23′ E), which is located at an altitude of 2,243 m absl. Gillnet surveys were carried out monthly between August 2011 and June 2012 using multifilament gillnet fleets comprising 60, 80, 100 and 120 mm stretched mesh size made from 210 D/3 twine. The panel length of each mesh size was 25 m with 3 m depth. Immediately after capture, the total length (T_L) and total weight (T_W) were measured and weighted to the nearest 1 mm and 0.1 g, respectively. Fish were then dissected, sexed and the gonads were assigned a stage of maturity according to Ref. [13].

2.2 Data Analysis

Sex ratio was computed and chi-square (χ^2) test was used to determine if it varied from the null hypothesis 1:1 [14]. The length-weight relationship was determined by using nonlinear least-squares regression (SPSS, 2011).

Gill net selectivity was estimated by using SELECT method [15]. The SELECT method applies maximum likelihood which estimates selectivity parameters from a general log-linear model [16]. The expected catch (V) of *Oreochromis niloticus* of length class i in gillnet j is described by:

$$V_{ij} = P_j \lambda_i r_j \quad (1)$$

where, P_j is the relative fishing intensity of gillnet j; λ_i is the abundance of *Oreochromis niloticus* in length class i; r_j is the selection curve for each gillnet j.

Relative fishing intensity represents fishing effort and fishing intensity combined and is the conditional probability that a fish contacted gillnet panel j with the assumption that it made single contact with the entire combined gillnet panel [12]. The normal, gamma and lognormal models (Table 1) observe geometric similarity (mean (μ_j) and spread (σ_j) proportional to mesh size) whereas the normal model with fixed spread is not geometrically similar (mean (μ_j) and spread (σ_j) equal across mesh sizes).

Catch data from Amerti reservoir were pooled by mesh size into 1 cm length classes, and the midpoint of each size class was used to estimate a selectivity curve for each mesh size. The four gillnet selectivity models (normal location, normal scale, gamma and log-normal) were fitted to the data by using the "gillnet functions" package in R statistical software [16, 17]. For each model, the data were fitted under the assumptions of equal effort and proportional effort to the size of the mesh. Goodness of fit statistics in the form of model deviance was used to choose the best model.

The mean length of fish at first maturity (L_{50}) was determined using the method described in Ref. [18]. The method fits the percentages of mature fish that were grouped in 1 cm length classes to the logistic equation:

$$P_L = (\exp(\alpha + \beta L))/(1 + \exp(\alpha + \beta L)) \quad (2)$$

where, P_L is proportion of mature fish at length L, L is total length (cm) and α (the intercept) and β (the slope) of least-squares estimates.

3. Results

A total of 657 *Oreochromis niloticus* specimens, comprising 398 males and 259 females were captured during the sampling period. The sex ratio was biased to males (χ^2 = 22.47, P < 0.0001). The T_L of the specimens ranged from 12.0 cm to 35.5 cm (Fig. 1) with a corresponding weight of 56.9-1,535.8 g. The length-weight relationships were curvilinear and statistically significant with $W = 0.016 L_T^{3.034}$, $R^2 = 0.98$.

Average length (± SD) of *Oreochromis niloticus* captured by the gill nets with 60, 80, 100 and 120 mm mesh size were 16.3 ± 1.5, 22.5 ± 2.8, 24.8 ± 1.8 and

Table 1 Selectivitycurves for normal, gamma and lognormal models used to estimate gillnet selectivity for *Oreochromis niloticus.*

Model	Selection curve	Modal length
Normal (fixed spread)	$\exp\left[-\frac{(l_j - k \cdot m_i)^2}{2\sigma^2}\right]$	$k \cdot m_i$
Normal	$\exp\left[-\frac{(l_j - k_1 \cdot m_i)^2}{2k_2^2 \cdot m_i^2}\right]$	$k \cdot m_i$
Gamma	$\frac{m_i}{l_j m_1} . \exp\left\{\mu - \frac{\sigma^2}{2} - \frac{\left[\log(l_j) - \mu - \log(m_i/m_1)^2\right]}{2\sigma^2}\right\}$	$(\alpha - 1)km_i$
Lognormal	$\left[\frac{l_j}{(\alpha-1)km_i}\right]^{\alpha-1} . \exp\left(\alpha - 1 - \frac{l_j}{km_i}\right)$	$\exp(\mu - \sigma^2)\left(\frac{m_j}{m_1}\right)$

where, l_j is the midpoint of length class j; m_i is the mesh size for panel i (i = 1-4 panels).

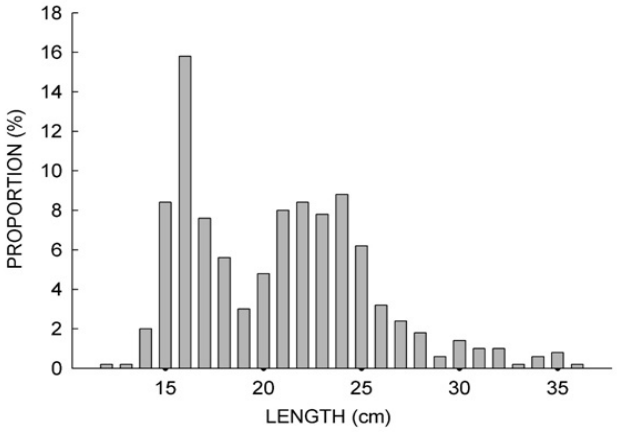


Fig. 1 Length frequency distribution of captured *Oreochromis niloticus* in Amerti reservoir.

30.3 ± 4.4 cm, respectively (Fig. 2). The size at first sexual maturity (L_{50}) for male *Oreochromis niloticus* was 21.5 cm T_L while the females attained L_{50} at 18.9 cm T_L (Fig. 3). The smallest female found with ripe gonads was 14.9 cm T_L. Majority of the individuals (99.5%) captured by 60 mm mesh size were below L_{50} (Fig. 2). There was a general increase in the mean size of *Oreochromis niloticus* with increasing mesh size.

There is no significant difference among the modes (i.e., lengths at maximum selectivity) estimated for each mesh size across all models (Fig. 4). However, the lognormal models with effort either equal or proportional to mesh size had identical and the lowest model deviance (Table 2) among the four models fitted using the SELECT method. The parameters and model variance of the selectivity curves of the normal scale, normal location, log normal and gamma models

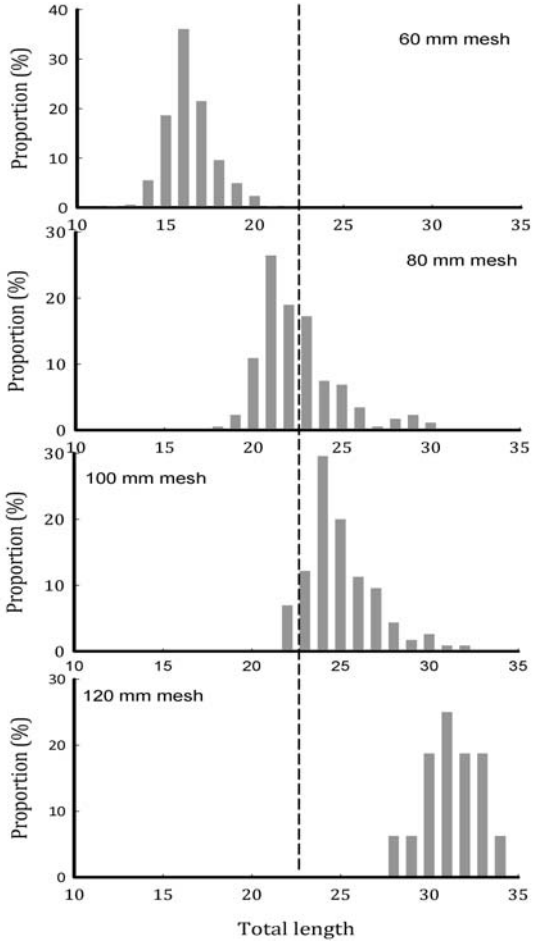


Fig. 2 Proportion of *Oreochromis niloticus* captured by (60-120 mm) mesh sizes gillnet. Dotted line indicates size at first maturity.

are shown in Table 2. The modes of the lognormal selection curves was found at 16.66, 22.26, 27.78 and 33.38 cm T_L for the 60, 80, 100 and 120 mm mesh sizes, respectively (Fig. 4).

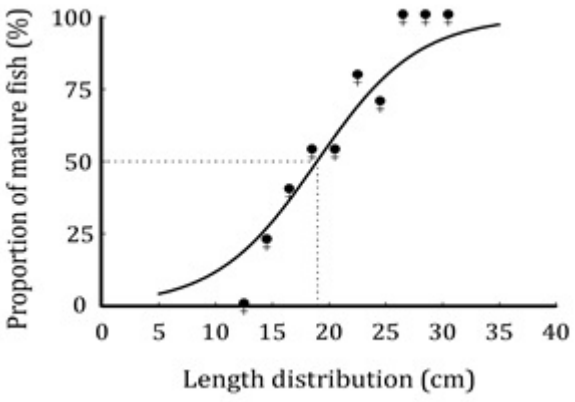


Fig. 3 Length at first maturity of female *Oreochromis niloticus* in Amerti reservoir.

4. Discussion

For Amerti reservoir, the catch was biased towards males in the current study (1.54:1). Slight variation in sex ratio of wild population is a typical phenomenon among Tilapias [4, 19] which might be attributable to differential migration of sexes [20].

The size at first maturity for *Oreochromis niloticus* is variable and broad in range in several East African lakes and reservoirs, ranging from 13 cm, Lake Tanganyika [21] to 42 cm, Lake Chamo [22]. Size at maturity in *Oreochromis niloticus* is inversely related

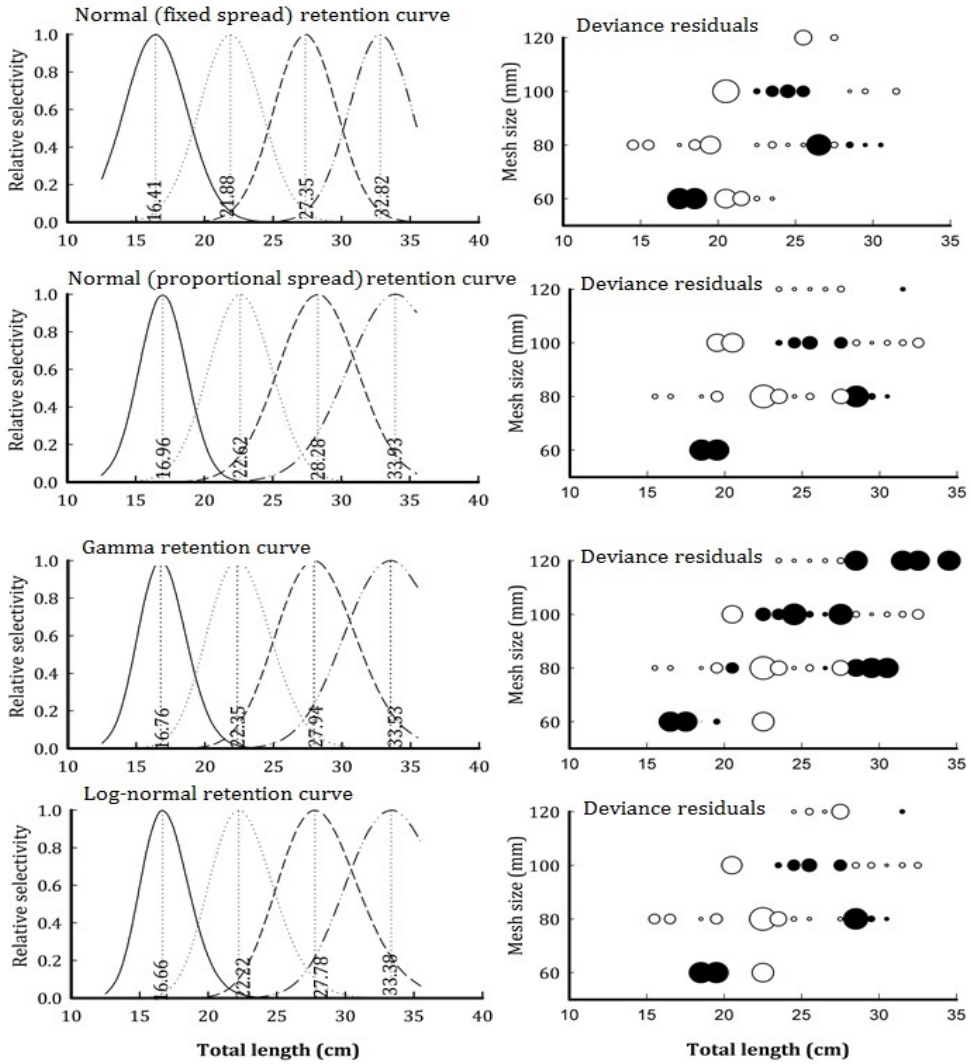


Fig. 4 Gillnet selectivity curves and residuals estimated for *Oreochromis niloticus* in Amerti reservoir for the 60-120 mm mesh sizes.

The plots on the left are the estimated gillnet selectivity curves while the plots on the right show the residuals of the models. Filled circles represent positive residuals and open circles represent negative residuals. The area of the circle is proportional to the square of the residual.

Table 2 Results of the models fit using the SELECT method for gillnet selectivity estimation, *Oreochromis niloticus* in Amerti reservoir.

Model	Equal fishing power		Fishing power α mesh size	
	Parameters	Deviance	Parameters	Deviance
Normal (fixed spread) (k, σ)	(0.274, 2.812)	105.08	(0.28, 2.29)	109.47
Normal (k_1, k_2)	(0.28, 0.0002)	98.45	(0.29, 0.0008)	98.60
Gamma (α, k)	(93.53, 0.003)	86.88	(95.4, 0.003)	86.88
Lognormal (m_1, σ)	(2.82, 0.104)	82.45	(2.84, 0.104)	82.45

to growth, and thereby is influenced by ecological habitat [23] and fishing pressure [3-5]. The latter could be ruled out for Amerti reservoir as there is no well-established fishing in the reservoir.

The model deviances obtained under the two assumptions about fishing power were essentially equal, and residual plots (not shown) were nearly identical. The ratio of model deviance to degrees of freedom was slightly higher than 1 (1.2), indicating slight over dispersion of the data. This result indicates that Nile tilapia may not have behaved independently violating the first assumption of independent catches. However, over dispersion does not necessarily affect parameter estimation [11, 12].

Deviance plot showed a similar degree of bias towards all models except the gamma model which showed the highest number of positive residuals for the higher length classes (22-34 cm T_L) in mesh sizes 80, 100 and 120 mm (Fig. 4). The plot indicated that more of the larger individuals were caught in these mesh groups than predicted by the models. The 80 mm mesh sizes caught fewer of the smallest *Oreochromis niloticus* than predicted by the lognormal model. The smallest mesh size (60 mm) panel caught more smaller (< 20 cm T_L) *Oreochromis niloticus* than predicted (Fig. 4).

In general, the gillnet with mesh size 80 mm most efficiently selects individuals about 17-30 cm in length, and the selection has a sharp peak in about 22 cm length class. Length at first maturity has a great importance in the determination of optimum mesh size [24]. The estimated lengths for females and males of *Oreochromis niloticus* in this study were 18.9 cm T_L and 21.5 cm T_L, respectively. Fishing gear restriction is a preferable way of management than closure in small scale artisanal fisheries [9] as it does not threaten the livelihoods of fishers which depend only in fisheries. Thus, the legal minimum size for *Oreochromis niloticus* should be 21 cm which can be achieved if the minimum mesh size is established at 80 mm.

Acknowledgments

The author's sincere gratitude goes to the technical staff of Ziway Fisheries Resources Research Center, particularly Mr Getachew Senbete, Mitiku Bonxa and Dawit Imiru for their assistance during the fieldwork. The study was financed by the Oromia Agricultural Research Institute (OARI).

References

[1] E. Trewavas, Tilapiine Fishes of the Genera *Sarotherodon*, *Oreochromis* and *Danakilia*, British Natural History Museum, London, 1983, p. 583.

[2] R.L. Welcome, International introductions of inland aquatic species, FAO Fish Tech Pap 294, 1988, p. 318.

[3] J. Chervinski, Environmental physiology of tilapias, in: The Biology and Culture of Tilapias, 1982, pp. 119-128.

[4] M. Njiru, J.E. Ojuok, J.B. Okeyo-Owuor, M. Muchiri, M.J. Ntiba, I.G. Cowx, Some biological aspects and life history strategies of Nile tilapia *Oreochromis niloticus* (L.) in Lake Victoria, Kenya, Afr. J. Ecol. 44 (2006) 30-37.

[5] J.E. Ojuok, M. Njiru, M.J. Ntiba, K.M. Mavuti, The effect of overfishing on the life-history strategies of Nile tilapia, *Oreochromis Niloticus* (L.) in the Nyanza Gulf of Lake Victoria, Kenya, Aquat. Ecosyst. Health Manag. 10 (2007) 443-448.

[6] D. Admaasu, Maturity, fecundity, brood-size and sex ratio of tilapia (*Oreochromis niloticus*) in Lake Awassa (Ethiopian Rift Valley), Hydrobiologia 337 (1996) 77-83.

[7] D. Reyntjens, T. Wudneh, Fisheries management: A review of the current status and research needs in Ethiopia, SINET: Ethiop. J. Sci. 21 (1988) 231-266.

[8] A.K. Morison, Input and output controls in fisheries management: A plea for more consistency in fisheries management, Fish. Manag. Ecol. 11 (2004) 411-413.

[9] T.R. McClanahan, J. Maina, J. Davies, Perceptions of resource users and managers towards fisheries management options in Kenyan coral reefs, Fish. Manag. Ecol. 12 (2005) 105-112.

[10] R.B. McAuley, C.A. Simpfendorfer, I.W. Wright, Gillnet mesh selectivity of the sandbar shark (*Carcharhinus Plumbeus*): Implications for fisheries management, ICES J. Mar. Sci. 64 (2007) 1702-1709.

[11] R.B. Millar, R.J. Fryer, Estimating the size-selection curves of towed gears, traps, nets and hooks, Rev. Fish Biol. Fish. 9 (1999) 89-116.

[12] I.E. Baremore, D.M. Bethea, K.I. Andrews, Gillnet selectivity for juvenile blacktip sharks (*Carcharhinus Limbatus*), Fishery Bulletin 110 (2012) 230-241.

[13] T.B. Bagenal, E. Braum, Eggs and early life history, in: Methods for Assessment of Fish Production in Freshwaters, IBP Handbook No. 3, 1968, pp. 165-201.

[14] J.H. Zar, Biostatistical Analysis, Prentice-Hall, New Jersey, 1999, p. 620.

[15] B.M. Russell, H. Rene, Estimation of gillnet and hook selectivity using log-linear models, ICES J. Mar. Sci. 54 (1997) 471-477.

[16] R.B. Millar, R code for fitting select models to gillnet data, http://www.stat.auckland.ac.nz/~millar/selectware/R/gillnets/ (accessed Aug. 10, 2013).

[17] R Development Core Team, A programming environment for data analysis and graphics, http://www.R-project.org (accessed Aug. 10, 2013).

[18] T.W. Echeverria, Thirty-four species of California rockfishes: Maturity and seasonality of reproduction, U.S. Fish. Bull. 85 (1987) 229-250.

[19] C.N.J. Luís, D.C. Edmir, Reproduction, food dynamics and exploitation level of *Oreochromis niloticus* (Perciformes: Cichlidae) from artisanal fisheries in Barra Bonita Reservoir, Brazil, Rev. Biol. Trop. 60 (2012) 721-734.

[20] J.N. Rinne, B. Wanjala, Observations on movement patterns of *Tilapia* spp. in Nyanza Gulf, Lake Victoria, East Africa, J. Fish Biol. 20 (1982) 317-322.

[21] N. Mulimbwa, O. Sarvala, M. van der Knaap, The effect of intensive fishing on two cichlids (*Oreochromis niloticus* and *Astatotilapia burtoni*) in Nyangara wetland at the north end of Lake Tanganyika (D. R. Congo) with fishery management implications, Aquat. Ecosyst. Health Manag. 13 (2010) 11-19.

[22] Y. Teferi, D. Admaasu, S. Mengistou, Breeding season maturation and fecundity of *Oreochromis niloticus* L. (Pisces: Cichilidae) in Lake Chamo, Ethiopia, SINET: Ethiop. J. Sci. 24 (2001) 255-264.

[23] R.H. Lowe-McConnell, Tilapias in fish communities, in: The Biology and Culture of Tilapias, Manila, Philippines: ICLARM Conference Proceedings, 1982, pp. 309-316.

[24] E.O. Caner, B.K. Volkan, M. Mavruk, A. Erhan, Spawning, maturity length and size selectivity for pikeperch (*Sander lucioperca*) in Seyhan Dam Lake, J. Vet. Anim. Sci. Adv. 10 (2011) 545-551.

Journal of Agricultural Science and Technology A 4 (2014) 141-148
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Lethal and Sublethal Effects of Leaves Extracts from Native and Exotic Plants in Southern Brazil against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) Caterpillars

Marianna Pilla D'Incao, Bárbara Cravo de Quadros, Paula Soares, Neiva Knaak and Lidia Mariana Fiuza
Laboratório de Microbiologia e Toxicologia, PPG em Biologia, Universidade do Vale do Rio dos Sinos (UNISINOS), São Leopoldo, Rio Grande do Sul, CEP. 93022-000, Brasil

Received: October 16, 2013 / Published: February 20, 2014.

Abstract: The caterpillar *Spodoptera frugiperda* is an important pest of several crops, due to the damage it causes and the difficulty of its control. The method of controlling these pests is through chemical insecticides, which cause adverse effects to the biotic and abiotic environment. The selection of new insecticides that meet the requirements of efficacy, safety, selectivity and those are economically viable, is highlighting the study of mechanisms of plant defense. The present study aimed to verify the insecticide effect of 27 plants in three different types of plants extracts: aqueous, decoction and polypeptide on *S. frugiperda*. Three plants were chosen for performing sublethal effects bioassays: two plants with the highest corrected mortality (*E. pulcherrima* and *R. simsii*) and the other plant (*Maytenus ilicifolia* (espinheira santa)) with corrected mortality similar to control. In preliminaries bioassays, 25 plants showed corrected mortality less than 50%, according to Abbott's formula. *R. simsii* (azalea) and *E. pulcherrima* (billed parrot) showed 51% and 68% of corrected mortality, respectively. Only *R. simsii* aqueous and the three extracts of *E. pulcherrima* differ significantly from control ($P < 0.05$). The aqueous extracts and decoction of *R. simsii* and polypeptide extract of *E. pulcherrima* reduced the larval period of *S. frugiperda*, different from control ($P < 0.05$). The polypeptide extract of *R. simsii* reduced the pupal period of the target insect. The extracts obtained by decoction, aqueous and polypeptide of *E. pulcherrima* extracts and decoction of polypeptides and *R. simsii* affected the fertility and fecundity of *S. frugiperda*. For the other parameters, there is no significant difference when compared with the control. The results of this study suggest that the extracts obtained by decoction, aqueous and polypeptide of *E. pulcherrima* and *R. simsii* can be used to control *S. frugiperda*. However, the successful search, product starting from extracts of plants depends on the availability of plant and the entire assembly around the detection of bioactive products, in addition to the active ingredients of the chemical synthesis and elucidation of the target site insecticide molecule.

Key words: Armyworm, *Spodoptera frugiperda*, plant extracts, bioassays, biopesticide.

1. Introduction

The caterpillar *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is an important pest for several crops such as corn (*Zea mays*), rice (*Oryza sativa*), soybeans (*Glycine max*), peanuts (*Arachis hypogaea*) and sorghum (*Sorghum bicolor*), and it is considered as one of the most destructive pests of agriculture worldwide among others [1, 2]. Currently, the most commonly used method to control these pests are synthetic insecticides which yields effective results in a short time, but the development of resistance to these insecticides by insects is also very fast, leading farmers to increase dosages or change the active principle most frequently [3-5]. Brazil is the largest consumer of pesticides in the world, with one third of

Corresponding author: Marianna Pilla D'Incao, Ph.D. candidate, research field: biological control. E-mail: maripdincao@gmail.com.

the food consumed by Brazilians which is contaminated by these substances, e.g., acephate and glicosato. Over the past decade, while the global consumption of pesticides has increased by 93%, the Brazilian has increased 190% in the use of pesticides, surpassing the consumption in the United States. In 2010, Brazil spent approximately US $7.3 billion in the purchase of pesticides, including insecticides, herbicides, fertilizers, fungicides and bactericides, among others [6].

The use of pesticides causes many adverse effects to biotic and abiotic environment, such as accumulation of residues in soil, water, air and consequently, animals and plants, including humans [4, 7, 8]. In this context, the selection of new insecticide molecules, obtained from vegetable raw materials, offers a wide range of new sites of action on target organisms which meet the requirements of efficacy, selectivity and safety, and sources of substances that can be used to develop safe methods to control insects.

The use of plant extracts for the control of pests in agriculture is very old, and because of the search for environmentally friendly practices, the use of plants with insecticidal properties is increasing [5, 8-11].

The use of substances extracted from plants as insecticides has numerous advantages compared to the use of synthetic, because they are quickly renewable, biodegradable, and do not persist in the environment. The products obtained starting from plants, by presenting a diversity of active ingredients, may present less risk of resistance to the target pests. Bermúdez-Torres et al. [12] reported 83% mortality of *S. frugiperda* when exposed to alkaloid extract *Lupinus stipulates* J. Agardh at a dose of 25 µg/mL. Alves et al. [13] achieved a 60% mortality of *S. frugiperda* after 7 d of exposure to copaiba leaf extract (*Copaifera langsdorffii* Desf. (Fabales: Fabaceae)). Rossi et al. [14] exposed larvae of *S. frugiperda* to the *Ricinus communis* L. (Malpighiales: Euphorbiaceae) 10% extract and obtained 89.9% mortality. Muñoz et al. [15] observed 58.3% mortality by exposing larvae of *S. frugiperda* to *Calceolaria talcana* J. Grau & C. Ehrh (Lamiales: Calceolariaceae) extracts.

Thus, the present study aimed to identify the lethal and sublethal effects of leaf extracts of three plants previously selected through screening of 27 native and exotic plants against *S. frugiperda* polyphagous species. The constant search for new methods of control, such as the use of new active principles derived from plants, which can be used seamlessly with the other methods, accounts of important assumptions Integrated Pest Management (IPM), reducing the impact of the use of chemicals.

2. Materials and Methods

2.1 Rearing and Conservation of S. frugiperda

The *S. frugiperda* used in the experiments were obtained from a pre-established population of species in insect rearing room of the Laboratory of Microbiology and Toxicology, University of Vale do Rio dos Sinos—UNISINOS, São Leopoldo, RS, Brazil.

The larva was fed with artificial diet described by Poitout and Bues [16] at larval stage, and in adulthood, with a solution of 10% sucrose. The life cycle was developed under controlled conditions of 25 ± 2 °C, 12 h photoperiod and 70% relative humidity.

The sampling of *S. frugiperda* in the field was carried out in the irrigated rice crops with the collaboration of researchers from the Instituto Rio Grandense do Arroz (IRGA) in the Estação Experimental do Arroz (EEA), in Cachoeirinha, RS, Brazil.

2.2 Plants Extracts

The leaves of native and exotic plants (Table 1) were collected on the campus of the University of Vale do Rio dos Sinos/UNISINOS (29°47′47,90″S and 51°09′24,93″WGr) in São Leopoldo, RS, Brazil. The chemical composition of plants can be altered by various factors, thus the collection of samples was

Table 1 Native and exotic plants collected on the campus of UNISINOS, São Leopoldo, RS, Brazil, for assessing the potential insecticide.

Scientific names	Families	Active ingredients
Aloe ferox	Aloeaceae	Antracenic compounds[1]
Aloe vera	Aloeaceae	Antracenic compounds[1]
Anchieta salutaris A. St.-Hil.	Violaceae	Alkaloids[5]
Artemisia camphorate Vill.	Asteraceae	Terpens[1]
Casearia sylvestris Sw.	Flacourtiaceae	Flavons, saponins, tannins[1]
Cinnamomum zeylanicum Blume	Lauraceae	Phenolic compounds[3]
Cordia monosperma (Jacq.) Roem. & Schult.	Boraginaceae	Flavonoids[1]
Equisetum arvense L.	Equisetaceae	Cumarins, alkaloids[6]
Eugenia uniflora L.	Myrtaceae	Tannins[1]
Euphorbia milii des Moulins	Euphorbiaceae	Diterpens[1]
Euphorbia pulcherrima Willd.	Euphorbiaceae	Diterpene esters[1]
Ficus pumila L.	Moraceae	Furanocumarins[1]
Ginkgo biloba L.	Ginkgoaceae	Flavonoids[1]
Jodina rhombifolia (Hook & Ain)	Santalaceae	Tannicacid, flavonoids[3]
Maytenus ilicifolia (Schrad.) Planch	Celastraceae	Poliphenols[2]
Mikania laevigata Sch. Bip. ex Backer	Asteraceae	Poliphenols, tannins e cumarins[1]
Nerium oleander L.	Apocynaceae	Cardenolids[1]
Pachystroma longifolium (Nees) I.M. Johnst	Asteraceae	Flavons, labdanditerpene, phenols e clerodans[4]
Pereskia grandifolia Haw.	Cactaceae	Alkaloids[7]
Phyllanthus niruri L.	Phyllanthaceae	Flavonoids[8]
Piper umbellata	Piperaceae	Terpens[1], phenols[6]
Plectranthus neochilus Schltr.	Lamiaceae	Quinones[1]
Rhododendron simsii Planch.	Ericaceae	Diterpens[1], graianotoxins[2]
Schinus molle L.	Anacardiaceae	Uruchols[1]
Schinus terebinthifolius Raddi	Anacardiaceae	Uruchols[1]
Solanum sisymbriifolium Lam.	Solanaceae	Alkaloids[1]
Syzygium cumini (L.)	Myrtaceae	Phenylpropanoid[2]

[1]Simões et al. [17]; [2]Lopes et al. [18]; [3]Pinto et al. [19]; [4]Ferrante et al. [20]; [5]Santos [21]; [6]Kaziyama et al. [22]; [7]Souza et al. [23]; [8]Maia-Almeida et al. [24].

standardized, i.e., all samples were collected between 8 o'clock and 9 o'clock in the morning with no rain, flowering and fruiting.

2.3 Screening of Plants and Solvents for Secondary Metabolites Extraction

For this study, 27 native and exotic plants collected on the campus of UNISINOS, possessing ethnobotanical record, scientific reference or known in the popular culture of the State of Rio Grande do Sul, Brazil, were selected.

Plant extracts were pre-selected through preliminary bioassays. Second instar larvae of *S. frugiperda* were maintained in individual mini acrylic plates (35 mm diameter) containing a thin layer of agar gel and a hard sheet (*Neonotonia wightii*), with 18 mm diameter, containing 50 μL of extracts. Two controls were performed: one with the solvent used in the extraction and the other with sterile distilled water. Each treatment consisted of 30 tracks with three replications.

The treatments were kept in a climatic chamber at 25 °C, 70% relative humidity and photoperiod of 12 h. The mortality was checked on day seven after treatment application, and then was corrected using Abbott's formula [25].

2.4 Obtaining Aqueous and Proteic Plant Extracts

The plant extracts were obtained by maceration of

leaves with liquid nitrogen to obtain a fine powder. The re-suspension of this powder was performed with five different solvents for the extraction of secondary metabolites, in a ratio of 1w:5v (5 mL of solvent for each plant powder gram) and kept 24 h at 4 °C, and subsequently filtered through sterile filter paper. After filtration, the extract was divided into tubes with the amount used in each test and kept at -18 °C.

Five extracts were prepared: cold aqueous extract (aqueous), hot aqueous extract (decoction), alcoholic extract (70% alcohol), methanol extract and an extract with buffer solution for extraction of polypeptides (50 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0; 5% glycerol v:v; 1 mM DTT; 0.1% Triton).

2.5 Bioassays for Determination of Acute Effect

From the results obtained in preliminary tests, three plants were selected to conduct acute bioassays: two plants with the highest corrected mortality (*E. pulcherrima* and *R. simsii*) and the other plant (*M. ilicifolia*) with corrected mortality similar to the control. Second instar larvae of *S. frugiperda* were maintained in individual mini acrylic plates (35 mm diameter) containing a thin layer of agar gel and a hard sheet (*Neonotonia wightii*) with 18 mm diameter containing 50 µL of extracts. Two controls were performed: one with the solvent used in the extraction and the other with sterile distilled water. Each treatment consisted of 30 tracks with three replications.

2.6 Bioassays for Estimate the Sublethal Effect

The tests for the sublethal effects of botanical extracts for *S. frugiperda* were performed by transferring all the surviving worms in bioassays for acute effect to disposable cups with artificial diet without the addition of treatments, which were monitored individually until the F1. The insects were kept in a room at 25 °C, 70% relative humidity and photoperiod of 12 h. Morphological changes, weight and length of the larval, pupal development, sex ratio and fertility were evaluated. Pupae were weighed on an analytical balance and measured with analogical pachymeter on the second day after molting.

2.7 Statistical Analysis

Statistical analysis was performed with the aid of the Systat 12 computer program. The mortality values obtained in bioassays were corrected by Abbott's formula [25] and then subjected to analysis of variance (ANOVA) followed by Tukey test ($P < 0.05$). The values of other parameters were also subjected to ANOVA followed by Tukey test ($P < 0.05$).

3. Results

The results of preliminary bioassays and the 27 plants used are described in Table 2, where 25 plants caused corrected mortality less than 50%. *R. simsii* and *E. pulcherrima* showed 51% and 68% of corrected mortality, respectively. Three plants were chosen to carry out bioassays: two plants with the highest corrected mortality (*E. pulcherrima* and *R. simsii*) and the other plant with low corrected mortality (*M. ilicifolia*).

In Table 3, the data presented correspond to the three plants chosen for the analysis of larval lethality and sublethal effects throughout the life cycle of *S. frugiperda*.

The results obtained in mortality bioassays were 6% for the control, 45.94% for *R. simsii* aqueous, 21.3% for *R. simsii* decoction, 15.2% for *R. simsii* polypeptide, 65.2% for *E. pulcherrima* aqueous, 64.47% for *E. pulcherrima* decoction, 76.11% for *E. pulcherrima* polypeptide, 7.33% for *M. ilicifolia* aqueous, 12.62% for *M. ilicifolia* decoction and 4.73% for *M. ilicifolia* polypeptide. The three extracts of *E. pulcherrima* differed significantly from all the other extracts with the exception of the aqueous extract of *R. simsii* ($P < 0.05$) (Table 3). The aqueous extract of *R. simsii* differed significantly only of aqueous and polypeptide extract of *M. ilicifolia* ($P < 0.05$) (Table 3).

Table 2 Screening data of potential insecticide leaf extracts from 27 native and exotic plants against second instar larvae of *S. frugiperda*.

Plants	Corrected mortality (%)		
Scientific name	Aqueous	Decoction	Polypeptide
Aloe ferox	20.0	28.9	11.1
Aloe vera	2.2	22.2	2.2
Anchieta salutaris	15.6	2.2	4.4
Artemisia camphorate	8.9	22.2	2.2
Casearia sylvestris	13.3	20.0	28.9
Cinnamomum zeylanicum	6.7	4.4	11.1
Cordia monosperma	4.4	13.3	0.0
Equisetum arvense	20.0	13.3	0.0
Eugenia uniflora	11.1	6.7	20.0
Euphorbia milii	2.2	6.7	13.3
Euphorbia pulcherrima	22.2	17.8	68.9
Ficus pumila	6.7	13.3	0.0
Ginkgo biloba	8.9	17.8	6.7
Jodina rhombifolia	4.4	46.7	11.1
Maytenus ilicifolia	6.7	17.8	0.0
Mikania laevigata	2.2	31.1	8.9
Nerium oleander	11.1	11.1	17.8
Pachystroma longifolium	6.7	4.4	2.2
Pereskia grandifolia	17.8	11.1	2.2
Phyllanthus niruri	6.7	4.4	20.0
Piper sp.	2.2	17.8	8.9
Plectranthus neochilus	4.4	6.7	15.6
Rhododendron simsii	11.1	31.1	51.1
Schinus molle	8.9	20.0	15.6
Schinus terebinthifolius	13.3	2.2	22.2
Solanum sisymbriifolium	13.3	13.3	4.4
Syzygium cumini	11.1	4.4	8.9

Table 3 Effect data of potential sublethal pesticide plant extracts, three native and exotic plants against larvae of second instar *S. frugiperda*.

Extrats	Corrected mortality (%)	Development time (d)			Measures (pupae)		Adults (nº)			Fertility and fecundity (Nº)		
		Larva	Pre-pupae	Pupae	Weight (g)	Length (mm)	Males	Females	Total	Couples	Postures	Postures ecloded
Control		20.99[a]	1.33	10.46	0.2135	15.58	10.4	10.2	20.6	6.8	6.4	4.8
Rhododendron simsii (A)	45.94[a]	12.83[b]	1.63	8.82	0.2049	14.58	5.4	5.2	10.6	4	3	2
Rhododendron simsii (D)	21.30[b]	17.57[b]	1.25	10.01	0.2094	15.35	5.2	5.4	10.6	3.4	2.6	1.2
Rhododendron simsii (P)	15.20[b]	19.4[a]	1.5	6.43	0.2159	15.04	8.4	8	16.4	5.8	4.8	3.2
Euphorbia pulcherrima (A)	65.20[a]	13.02[a]	1.42	10.85	0.2254	16.18	5.75	5	10.5	4	3.25	2.25
Euphorbia pulcherrima (D)	64.47[a]	13.73[a]	1.23	9.3	0.2190	16.22	3.8	4	7.8	2.6	2.2	1.8
Euphorbia pulcherrima (P)	76.11[a]	10.43[b]	1.24	9.39	0.2344	15.94	3	1	4	0.8	0.4	0.2
Maytenusilicifolia (A)	7.33[b]	20.53[a]	1.67	9.80	0.1970	14.84	9.8	9	18.8	5	4	3
Maytenusilicifolia (D)	12.60[b]	18.97[a]	1.12	9.94	0.1997	15.26	8.2	7.4	15.2	5.6	4.2	2.4
Maytenusilicifolia (P)	4.73[b]	19.47[a]	1.78	10.12	0.2376	15.72	13	11.2	24.2	9.2	7.6	5

A = aqueous, D = decoction, P = polypeptide;
In the same column, means with the same letter do not differ significantly ($P > 0.05$).

The larval period in control was approximately 21 d. *M. ilicifolia* aqueous showed similar larval duration (20.5 d), and *E. pulcherrima* polypeptide had the lowest larval period of about 11 d.

In control, the pre-pupal period was 1.33 d. The extract which exhibited the shortest duration in this period was *M. ilicifolia* decoction (1.12 d) and the longest was *M. ilicifolia* polypeptide (1.78 d).

The aqueous extract of *E. pulcherrima* showed pupal period similar to control (*E. pulcherrima*: 10.85 d and control: 10.46 d). The shortest pupal period was recorded in *R. simsii* polypeptide extract (6.43 d).

The weight of larvae in the control group was 0.2135 g, the lowest weight was recorded in the treatment with *M. ilicifolia* aqueous (0.1970 g) and the treatment with the highest weight was *M. ilicifolia* polypeptide (0.2376 g). In contrast to these data, the smallest pupae were observed in *R. simsii* aqueous treatment (14.58 mm) and the largest were recorded in the *E. pulcherrima* decoction extract (16.22 mm). The mean size in control pupae was 15.58 mm.

The total number of adults observed in the control treatment was 20.6 insects, with 10.4 males and 10.2 females, composing 6.8 couples. The treatment with the highest number of adults was *M. ilicifolia* polypeptide with 24.2 insects (13 males and 11.2 females) forming approximately 9.2 couples. The *E. pulcherrima* polypeptide extract showed the lowest number of adults in all repetitions (4 adults). There was a difference in the sex ratio of adults in this treatment, with three males and one female per repetition. It is not possible to form couples in all bioassays.

The number of couples that have made postures in the control group was 6.4, but only about 4.8 postures were hatched. The treatment with the lowest number of eggs was *E. pulcherrima* polypeptide, with 2.6 postures per repeat, which 0.2 erupted by bioassay. And the group which had the highest number of eggs was *M. ilicifolia* polypeptide with 7.6 postures per bioassays, where only 5 were hatched.

4. Discussions

In this study, the extracts of *E. pulcherrima* caused higher corrected mortality than *M. ilicifolia* and *R. simsii* extracts. The toxicity caused by extracts of this plant could be explained by the presence of phenolic compounds, such as quercetin and kaempferol [26-28]. Nenaah [29] reported mortality between 54% and 64% of *Sitophilus oryzae* (rice weevil) and *Rhyzopentha dominica* (buzzer on cereals), when fed with *Calotropis procera* (Ait.) (Gentianales: Asclepiadaceae) extracts, whose major secondary metabolites are phenolic compounds such as kaemferol. Moraes-Braga et al. [30] observed 68% mortality of *Trypanosoma cruzi* exposed to *Lygodium venustum* extract which presents kaempferol and quercetina as its main secondary metabolites.

The acute effect of aqueous extract of *R. simsii* may be due to the presence of diterpenes that are powerful deterrents to many species of insects [31]. These diterpenoid found in all species of *R. simsii*, are termed graianotoxins, toxins "alkaloid", and are bound to sodium channels in cell membranes increasing permeability of this ion in excitable membranes. This binding is reversible, but can cause serious damage to the affected cells [18-32]. Klocke et al. [33] obtained LC_{50} of 8.8 ppm by exposing *S. frugiperda* to the rhodojaponin-III, a type of graianotoxins extracted from *Rhododendron molle.*

D'Incao et al. [10] observed an increase in the larval period by exposing larvae of *S. frugiperda* to 312 ppm saponin extracted from *Passiflora alata* Curtis (1788) (Violales: Passifloraceae). In this work, the pre-pupa and pupa showed no significant difference in duration of treatment compared to the control, a result which confirms the results obtained by D'Incao et al. [10]. However polypeptide extract of *R. simsii* reduced the pupal period of *S. frugiperda*. The aqueous extracts and decoction of *R. simsii* and polypeptide extract of *E. pulcherrima* reduced the larval period of *S. frugiperda*, differing control and other treatments ($P < 0.05$).

In this study, the extracts obtained by decoction, aqueous and polypeptide of *E. pulcherrima* and decoction and polypeptide of *R. simsii* reduced the average number of eggs and hatched larvae, differing significantly from the control. Ramos-López et al. [34] observed a gradual decrease in pupal weight of *S. frugiperda* exposed to different extracts of *R. communis*.

The selection of plants with insecticidal activity is based on the lethal effects. However, it should be considered that the mortality of the insect requires higher dose, hence larger amount of vegetable raw material. Thus, the main objective should be to reduce or prevent the growth of the pest population, either by physiological effects and changes in sexual behavior or other related factors.

The results of this study suggest that the extracts obtained by decoction, aqueous and polypeptide of *E. pulcherrima* and *R. simsii* can be used to control *S. frugiperda*. However, the successful search, product starting from extracts of plants depends on the availability of plant and the entire assembly around the detection of bioactive products, in addition to the active ingredients of the chemical synthesis and elucidation of the target site insecticide molecule.

References

[1] S.J. Yu, S.N. Nguyen, G.E. Abo-Elghar, Biochemical characteristics of insecticide resistence in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), Pest. Bioch. Phys. 77 (2003) 1-11.

[2] A.R. Roel, J.D. Vendramim, Residual effect of the extract of ethyl acetate *Trichillia pallida* Swartz (meliaceous) for larvae of different ages *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), C. Rural 36 (2006) 1049-1054. (in Portuguese)

[3] L. Castillo, A. González-Coloma, A. Gonzáles, M. Días, E. Santos, E. Alonso-Paz, et al., Screening of Uruguayan plants for deterrent activity against insects, Ind. Crops Products 29 (2009) 235-240.

[4] N. Zapata, F. Budia, E. Viñuela, P. Medina, Antifeedant and growth inhibitory effects of extracts and drimanes of *Drimys winteri* stem bark against *Spodoptera littoralis* (Lep.: Noctuidae), Ind. Crops Products 30 (2009) 119-125.

[5] J.L. da Silva, R.N.V. Teixeira, D.I.P. Santos, J.O. Pessoa, Antifungal activity of plant extracts on the *in vitro* growth of pathogens, R. Verde 7 (2012) 80-86. (in Portuguese)

[6] F.F. Carneiro, W. Pignati, R.M. Rigotto, L.G.S. Augusto, A. Rizzolo, N.M. Muller, et al., Dossiê ABRASCO—Brazilian Association of Collective Health: A warning about the impact of pesticides on health, Part 1: Pesticides, Food Security and Health, World Nutrition, Rio de Janeiro, 2012. (in Portuguese)

[7] M. Rizwan-ul-Haq, Q.B. Hu, M.Y. Hu, Q.S. Lin, W.L. Zhang, Biological impact of harmaline, ricine and their combined effects with *Bacillus thuringiensis* on *Spodoptera exigua* (Lepidoptera: Noctuidae), J. Pest Science 82 (2009) 327-334.

[8] R. Pavela, M. Sajfrtová, H. Sovová, M. Bárnet, J. Karban, The insecticidal activity of *Tanacetum parthenium* (L.) Schultz Bip. extracts obtained by supercritical fluid extraction and hydrodistillation, Ind. Crops Products 31 (2010) 449-454.

[9] L.R. Bertholdo-Vargas, J.N. Martins, D. Bordin, M. Salvador, A.E. Schafer, N.M. de Barros, et al., Type 1 ribosome-inactivating proteins-entomotoxic, oxidative and genotoxic action on *Anticarsia gemmatalis* (Hübner) and *Spodoptera frugiperda* (J.E. Smith) (Lepidopera: Noctuidae), J. Insect Phys. 55 (2009) 51-58.

[10] M.P. D'Incao, G. Gosmann, V. Machado, L.M. Fiuza, G.R.P. Moreira, Effect of saponin extracted from *Passiflora alata* Dryander (Passifloraceae) on development of the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), Int. J. Plant Research 2 (2012) 151-159.

[11] N. Knaak, M.S. Tagliari, V. Machado, L.M. Fiuza, Insecticidal activity of extracts of medicinal plants on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), Bioassay 7 (2012) 1-6. (in Portuguese)

[12] K. Bermúdez-Torres, J.M. Herrera, R.F. Brito, M. Wink, L. Legal, Activity of quinolizidine alkaloids from three Mexican *Lupinus* against the lepidopteran crop pest *Spodoptera frugiperda*, BioControl 54 (2009) 459-466.

[13] D.S. Alves, G.A. Carvalho, D.F. Oliveira, R.R. Sâmia, M.A. Villas-Boas, G.A. Carvalho, et al., Toxicity of copaíba extracts to armyworm (*Spodoptera frugiperda*), African J. Biotech. 11 (2012) 6578-6591.

[14] G.D. Rossi, C.D. Santos, G.A. Carvalho, D.S. Alves, L.L.S. Pereira, G.A. Carvalho, Biochemical analysis of a castor bean leaf extract and its insecticidal effects against *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), Neotr. Entomol. 41 (2012) 503-509.

[15] E. Muñoz, C. Lamilla, J.C. Marin, J. Alarcon, C.L. Cespedes, Antifeedant, insect growth regulatory and insecticidal effects of *Calceolaria talcana* (Calceolariaceae) on *Drosophila melanogaster* and *Spodoptera frugiperda*, Ind. Crops Products 42 (2013) 137-144.

[16] S. Pouitout, R. Bues, Breeding of several species of Lepidoptera Noctuidae on artificial mitieu rich and simplified medium, Ann. Ecol. Anim 2 (1970) 79-91. (in French)

[17] C.M.O. Simões, E.P. Gosmann, G. de Mello, J.C.P. Mentz, L.A. Petrovick, Pharmacognosy: The Medicinal Plant, 6th ed., Editora UFRGS, Porto Alegre, 2007, p. 1102. (in Portuguese)

[18] R.K. Lopes, M.R. Ritter, S.M.K. Rates, Review of biological activities and toxicity of ornamental plants most used in Rio Grande do Sul, R. Bras. Biosciences 7 (2009) 305-315. (in Portuguese)

[19] A.C. Pinto, D.H.S. Silva, V. da S. Bolzani, N.P. Lopes, R.A. Epifanio, Natural products: Updates, challenges and prospects, Quím. Nova 25 (2002) 45-61. (in Portuguese)

[20] L.M.S. Ferrante, B. Mayer, E.C. Vasconcelos, C.M.R. Oliveira, GC/FID-based authentication of *Baccharis trimera*: A quality control study of products commercialized in Curitiba and metropolitan region (Brazil), Rev. Bras. Farmacog. 17 (2007) 356-360.

[21] N.P. dos Santos, Theodoro Peckolt: The scientific work of a pioneer in phytochemistry in Brazil, História, Ciências, Saúde 12 (2) (2005) 515-533.

[22] V.M. Kaziyama, M.J.B. Fernandes, I.C. Simoni, Antiviral activity of medicinal plant extracts against herpes commercially available porcine and bovine virus, Ver. Bras. Plantas Med. 143 (2012) 522-528. (in Portuguese)

[23] M.R.M. Souza, E.J.A. Correa, G. Guimarães, P.R.G. Pereira, The ora-pro-nobis potential as yield diversification to small farmers, Rev Bras. Agroecologia 4 (2) (2009) 3550-3554.

[24] C.I. Maia-Almeida, L.C. Ming, M.O.M. Marques, P.M. Magalhães, S.C.N. Queiroz, S. Scramim, et al., Density of planting and harvesting age of shatterstone (Phyllanthusamarus (Schumach. & Thonning) genotype-Unicamp CPQBA 14) in productivity phyllanthin, Rev. Bras. Plantas Med. 13 (2011) 633-641. (in Portuguese)

[25] W.S. Abbott, A method of computing the effectiveness of an insecticida, J. Econ. Entomol. 18 (1925) 265-267.

[26] M.P. Mariot, R.L. Barbieri, Secondary metabolites and medicinal properties of the thorn-santa (*Maytenus ilicifolia* Mart. ex. Reiss and *Maytenus aquifolium* Mart.), Bras. Plantas Med. 9 (3) (2007) 89-99. (in Portuguese)

[27] J. Alonso, C. Desmarchelier, *Maytenus ilicifolia* Martius (Cangorosa), Boletín Latino Americano y del Caribe de Plantas Med. y Aromáticas 6 (1) (2007) 11-22. (in Spanish)

[28] W.M.M. Jesus, T.N. da Cunha, Study of the pharmacological properties of the thorn-santa (*Maytenus ilicifolia* Mart. ex Reissek) and two species adulterants, Rev. S. e Des. 1 (1) (2012) 20-46. (in Portuguese)

[29] G.E. Nenaah, Potential of using flavonoids, látex and extracts from *Calotropis procera* (Ait.) as grain protectants against two coleopteran pests of stored rice, Experimental Paras. 134 (2) (2013) 178-182.

[30] M.F.B. Moraes-Braga, T.M. Souza, K.K.A. Santos, G.M.M. Guedes, J.C. Andrade, C. Veja, et al., Phenol composition, cytotoxic and anti-kinetoplastidae activities of *Lygodium venustum* SW. (Lygodiaceae), Biochemical Syst. Ecol. 42 (2012) 128-133.

[31] L.M. Schoonhoven, T. Jermy, J.J.A. Van Loon, Insect—Plant Biology: From Physiology to Evolution, Chapman & Hall, London, 1998, p. 409.

[32] G. Felippe, Poison: Plants That Kill and Also Heal, Editora Senac, São Paulo, 2009, p. 352. (in Portuguese)

[33] J.A. Klocke, M.Y. Hu, S.F. Chiu, I. Kubo, Grayanoid diterpene insect antifeedants and insecticides from *Rhododendron molle*, Phytochemistry 30 (1991) 1797-1800.

[34] M.A. Ramos-Lopéz, S. Pérez, C. Rodriguez-Hernández, P. Guevara-Fefer, M.A. Zavala-Sánchez, Activity of *Ricinus communis* (Euphobiaceae) against *Spodoptera frugiperda* (Lepidoptera: Noctuidae), African Journal of Biotechnology 9 (2010) 1359-1365.

Journal of Agricultural Science and Technology A 4 (2014) 149-154
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Comparative Utilization of Different Fibre Feedstuffs by Weaning/Growing Pigs in the Tropics

Emmanuel Oluropo Akinfala, Omotola Macaulay and Samuel Temitope Ogundeji
Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Osun State 220005, Nigeria

Received: May 23, 2013 / Published: February 20, 2014.

Abstract: This study was conducted to investigate the effect of feeding different fibre feedstuffs on the performance, nutrient utilization and economics of production of weaning/growing pigs. Five fibre feedstuffs—palm kernel cake (PKC), wheat offal (WO), corn bran (CB), rice bran (RB) and brewers' dried grain (BDG) were used at 25% in each of the five experimental diets that were formulated in this study. Twenty weaner pigs (12.75 ± 0.6 kg) were used for the growth study while 15 pigs (14.95 ± 0.57 kg) were used for the digestibility study. The design of the experiment was completely randomized. The results of the study showed that the performance of the experimental animals were significantly influenced ($P < 0.05$) by the dietary treatments. The apparent digestibility of the crude protein, crude fiber and ether extract showed significant differences ($P < 0.05$) while there was no significant difference ($P > 0.05$) in the apparent digestibility of their dry matter, ash and nitrogen free extract. The diet that contained WO had the best growth response and apparent nutrient digestibility. Results of economics of production also showed that diet with WO gave the best ($P < 0.05$) value in feed cost/kg gain and net profit/animal. It can be concluded from this study that although all the animals fed the different fibre feedstuffs performed satisfactorily on all the parameters monitored, but for optimum growth, apparent nutrients digestibility and economics of production. WO should be used as a fibre feedstuff in the diets of weaning/growing pigs in the tropics.

Key words: Pigs, performance, digestibility, economics of production, diets.

1. Introduction

Pig production according to Adesehinwa [1] represents the fastest means of correcting animal protein shortage in Africa. This is because, apart from their high rate of reproduction, poultry and pigs are characterized by the best efficiency of nutrient transformation into high quality protein (meat). For this reason, pig feeds and feeding must be efficiently managed to ensure the production of meat at the most economic level.

Due to serious competition between feed industry and other sources of food which has been resulting in high cost and scarcity of conventional feed ingredients [2] like maize, soybean meal, groundnut cake, fishmeal etc., most pig farmers in Nigeria use agro-industrial by-products such as palm kernel cake, wheat offal (WO), corn bran (CB), brewers' dried grain (BDG) and rice bran (RB) as basal feed ingredients. According to Amaefule et al. [3], there have been no evidence that these farmers who use these by-products to feed their pigs have sufficient knowledge of their nutritional value in terms of their nutrient content, digestibility and nutrient availability. Adesehinwa et al. [4] had recognized the use of agro-industrial by-products for livestock feeding especially in the developing countries as a measure for sustainable livestock development.

Although, the feeding value of these non-conventional feedstuffs had been documented [5-7] they may have undergone changes in their proximate and nutrient composition with time due to changes in the variety of crops and methods of industrial processing. For this reason, there will be the

Corresponding author: Emmanuel Oluropo Akinfala, Ph.D., research field: monogastric animal nutrition. E-mail: akinfala@oauife.edu.ng; oakinfala@yahoo.com.

need to always establish the bioavailability of nutrients from these fibre feedstuffs for pigs in order to ensure adequate feeding and at a low cost, too [3].

The use of these agro-industrial by-products as basal diets by most pig farms in South-Western Nigeria is presently not sufficient to support optimum productivity of the animals. The implication of this is that most of the animals cannot reach market age within a reasonable period of time. Although, there has been continuous investigation on the potentials of some agro-industrial by-products such as palm kernel cake (PKC), WO, RB, BDG etc., as feedstuffs for monogastric animals, the economics of their inclusion and which one is better utilized under the same management conditions have not been fully established.

Hence, this study was carried out to investigate the effect of feeding these different fibre feedstuffs on the performance, nutrient utilization and economics of production of weaning/growing pigs.

2. Materials and Methods

2.1 Location of Study and Source of Ingredients

The experiment was conducted at the Swine Unit of the Teaching and Research Farm of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. All the fibre feedstuffs were purchased from a commercial feedmill at Ibadan, Oyo State, Nigeria.

2.2 Experimental Pigs and Their Management

A total of 20 growing pigs of average weight 13.05 ± 1.08 kg were randomly assigned to five experimental diets. There were four animals per treatment and each of them served as a replicate. The experimental design was completely randomized. Routine management practices were carried on the animals on treatment basis. The experiment lasted for 42 d.

2.3 Apparent Nutrient Digestibility Study

The apparent nutrient digestibility experiment was carried out with 15 pigs with initial mean weight of 14.95 ± 0.57 kg. The pigs were randomly distributed into the five experimental diets that were formulated for this study. The pigs were put individually in a metabolism cage (107 cm × 60 cm × 50 cm) locally fabricated with metal for 7 d. The pigs were starved for 12 h before the commencement of the digestibility study to clear the gut of previous meals because markers were not used. The metabolic cages were equipped with watering and feeding facilities. They were fed 3% of their body weight while water was supplied *ad libitum.* Total excreta were collected daily during the last 4 d of the metabolic trial. The faeces was oven dried on daily basis at 60 °C, weighed and put in a labeled plastic bag and stored in a deep freezer for future use. At the end of these 4 d of fecal collection from each animal, the oven dried faeces was mixed, milled and representative samples were taken to proximate composition analysis.

2.4 Diets and Feeding

Five experimental diets with various fibre feedstuffs were formulated in this study. Diet 1 contained PKC, diet 2 contained WO, diet 3 contained CB, diet 4 contained RB and diet 5 contained BDG. All were included at 25% of the diets. All diets contained an average of 20% crude protein (Table 1). These diets were formulated to meet the requirements of growing pigs in the tropics.

Feed was allocated daily at 5% of their body weight and water was supplied *ad libitum* throughout the experimental period.

2.5 Data Collection

On daily basis, known and carefully measured feed was given to each animal and at the end of each week, feed remnant was weighed back and quantitative feed intake (feed disappearance) was calculated by difference. Records of weight gain, feed intake and feed to gain ratio were kept on treatment basis. The economics of production was determined using feed cost/kg gain by the animal and the net profit/each animal.

Table 1 Gross composition of experimental diets.

Ingredients (%)	Diets				
	1	2	3	4	5
Maize	45.0	45.0	45.0	45.0	45.0
Soybean meal	10.0	10.0	10.0	10.0	10.0
Groundnut cake	15.0	15.0	15.0	15.0	15.0
PKC	25.0	-	-	-	-
WO	-	25.0	-	-	-
CB	-	-	25.0	-	-
RB	-	-	-	25.0	-
BDG	-	-	-	-	25.0
Fish meal	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
*Premix	0.25	0.25	0.25	0.25	0.25
Bone meal	2.25	2.25	2.25	2.25	2.25
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Metabolizable energy (kcal/kg)	2,797.00	2,721.00	2,878.00	2,968.00	2,748.00
Crude protein (%)	21.25	21.50	19.50	19.80	21.30
Crude fibre (%)	5.32	4.32	5.32	4.45	7.32

*Grower premix supplied the following per kg diet: vitamin A 10,000,000 IU; vitamin D 32,000,000 IU; vitamin E 8,000 IU; vitamin K 2,000 mg; vitamin B1 2,000 mg; vitamin B2 5,500 mg; vitamin B6 1,200 mg; vitamin B12 12 mg; biotin 30 mg; folic acid 600 mg; niacin 10,000 mg; pantothenic acid 7,000 mg; choline chloride 500,000 mg; vitamin C 10,000 mg; iron 60,000 mg; Mn 80,000 mg; Cu 800 mg; Zn 50,000 mg; iodine 2,000 mg; cobalt 450 mg; selenium 100 mg; Mg 100,000 mg; anti-oxidant 6,000 mg.

2.6 Chemical and Statistical Analyses

The proximate composition of the fibre feedstuffs, diets and fecal samples were carried out using method outlined by Association of Official Analytical Chemists (AOAC) [8]. All the data collected were subjected to statistical analysis using a computer software package [9]. One-way analysis of variance (ANOVA) was used to compare the means while the means were separated using Duncan's multiple range test.

3. Results and Discussion

3.1 Proximate Composition of Test Ingredients

The proximate composition of test ingredients is presented in Table 2. The result showed that BDG had the highest value of crude protein 23.32% which was higher than 22.49% reported by Amaefule et al. [10]. The variation may be due to differences in source and processing methods. The crude fibre content ranged from 8.15% to 20.57% with BDG having the highest value while WO had the lowest value.

The proximate composition obtained for CB and RB had similarities with values reported by Aduku [11] and Kil et al. [12]. CB had the lowest percentage of ether extract (2.33%) while the highest value was found in RB. This may be due to monounsaturated fatty acids and low saturated fatty acids present in these feedstuffs [13].

3.2 Growth Performance

As shown in Table 3, the final live weight (kg) significantly differed ($P < 0.05$) across the five experimental diets. The highest value (27.13 kg) occurred in diet 2 while the lowest value (20.25 kg) occurred in diet 5. The average daily weight gain also showed significant difference ($P < 0.05$) across the five treatments. Diet 2 had the highest value (0.39 kg/d) while diet 5 had the lowest value (0.20 kg/d). This may be due to the better utilization of the nutrients in WO by the experimental animals than the other test ingredients. The highest feed intake (0.92 kg/d) was found in diet 1 ($P > 0.05$) while the lowest value was found in diet 5 (0.73 kg/d). The low feed intake in diet 5 may be due to the bulkiness of BDG which had been reported to have adverse effect on the

Table 2 Proximate composition of the test ingredients.

Proximate composition (%)	Fibre ingredients				
	1 (PKC)	2 (WO)	3 (CB)	4 (RB)	5 (BDG)
Moisture	90.24	90.74	89.40	89.20	89.90
Ash content	3.68	4.73	2.25	8.73	6.21
Crude fibre	10.54	8.15	10.55	11.63	20.57
Ether extract	6.72	4.48	2.33	13.14	3.25
Crude protein	22.53	18.81	12.69	13.56	23.32
Nitrogen free extract	56.53	63.83	81.18	52.94	46.65

Table 3 Growth and economics of production of the experimental animals.

Parameters	Diets					
	1	2	3	4	5	SEM
Initial body weight (kg)	13.50	12.88	13.25	12.88	12.75	0.04
Final body weight (kg)	26.00^{a}	27.13^{a}	23.63^{b}	24.13^{b}	20.25^{c}	1.54
Average daily weight gain (kg)	0.34^{b}	0.39^{a}	0.28^{c}	0.30^{b}	0.20^{d}	0.14
Average daily feed intake (kg)	0.92^{a}	0.90^{a}	0.85^{b}	0.84^{b}	0.73^{c}	0.11
Feed/gain ratio	2.72^{b}	2.38^{a}	3.01^{c}	2.90^{b}	3.65^{d}	0.23
Feed cost/kg of diet ($)	0.47	0.49	0.50	0.45	0.46	0.09
Feed cost/kg weight gain ($)	1.27^{b}	1.16^{a}	1.49^{c}	1.31^{b}	1.68^{d}	0.09
Net profit/animal ($)	26.45^{b}	30.22^{a}	21.89^{c}	24.32^{b}	16.18^{d}	2.35

a, b, c: means in the same row having different superscripts differ at $P \leq 0.05$;
₦150 = 1$.

digestibility of nutrients in any feed/feedstuffs [14]. This may be the reason for the observed growth response of the animals in this study. The weight gained by the animals in this study appeared to be influenced by the feed intake. The more the animal ate the more weight they gained. There were significant differences ($P < 0.05$) in the feed to gain ratio values across the treatment. Diet 2 had the best feed to gain ratio (2.38) while diet 5 had the worst (3.65).

The growth response of the experimental animals on WO and PKC based diets were similar. This may be due to their lower fibre contents compared to the other fibre feedstuffs used in this study. This according to Alawa and Umunna [15], make them more suitable for feeding monogastric animals. The poorer weight gain of animals on diet 5 may be due to high crude fibre level of BDG which affects digestibility [16].

3.3 Economics of Production

The economics of production of the experimental animals is shown in Table 3. The net profit/animal differ significantly ($P < 0.05$) with diet 2 generating the highest profit ($30.22) while diet 5 generated the least profit ($16.18). The feed cost/kg weight gain was the lowest ($P < 0.05$) in diet 2 ($1.16) and the highest ($1.68) for diet 5. The feed cost/kg of feed showed no significant difference ($P > 0.05$) across the five dietary treatments. Diet 4 had the lowest feed cost/kg ($0.45) while diet 3 had the highest value ($0.50). The variation in the cost of feed/kg was due to variation in cost of test ingredients. Although, diet 4 gave the least cost ration but it did not translate it into more profit from the animals on this diet. Moreover, despite the fact that diet 2 was not the cheapest diet, it gave the best economics of production indices in this study. The combination of improved weight gain and best feed conversion efficiency may have been responsible for higher returns in diet 2 compared to other diets. Also the protein in WO may be of higher quality, more digestible and palatable and in addition, more nutrients may have been made available compared with the other ingredients tested [17].

3.4 Apparent Nutrient Digestibility

The apparent nutrient utilization is shown on Table 4. There was no significant difference ($P < 0.05$) in the apparent digestibility value for ash and nitrogen free

Table 4 Apparent nutrient digestibility of the animals fed the experimental diets.

Parameters (%)	Diets					
	1	2	3	4	5	SEM
Dry matter	88.94	93.84	79.78	84.76	81.48	1.86
Crude protein	86.89[b]	94.22[a]	86.44[b]	88.23[b]	74.69[c]	2.08
Crude fibre	76.62[a]	78.63[a]	77.67[c]	67.03[b]	56.59[c]	5.79
Ether extract	85.70[b]	97.58[a]	69.16[b]	54.27[c]	55.65[c]	5.24
Ash	64.79	65.83	67.67	61.57	56.15	5.51
Nitrogen free extract	92.96	95.20	84.05	93.08	88.99	1.61

a, b, c: means in the same row having different superscripts differ at $P \leq 0.05$.

extract across all the treatments, while crude fibre and ether extract are significantly different ($P > 0.05$) across all the treatments. The highest crude protein digestibility (94.22%) was found in diet 2 while the least digestibility was found in diet 5 (74.69%) indicating that BDG was less digestible which may be as a result of its higher fibre content. Apparent crude fibre digestibility was significantly the highest ($P < 0.05$) in diet 2 (78.63%) while the least was diet 5 (56.59%). The highest ether extract value ($P < 0.05$) was found in diet 2 (97.58%) and the least was found in diet 4 (54.27%). The differences in the digestibility of the proximate components of the test ingredients may be due the level of fibre in them. It agreed with the findings of Jorgensen et al. [18] who identified fiber as one of the most important factors that affected digestibility in pigs. The lower fibre content of WO compared to PKC makes it more suitable for the feeding of monogastric animals [15]. Nonetheless, the proximate components of all the fibre feedstuffs based diets in this study appeared to be well digested. This may have been responsible for the acceptable growth rate of all the animals in this study.

4. Conclusions

It can be concluded from this study that although all the animals fed the different fibre feedstuffs performed satisfactorily on all the parameters monitored, but for optimum growth, apparent nutrients digestibility and economics of production, WO should be used as a fibre feedstuff in the diets of weaning/growing pigs in the tropics.

References

[1] A.O.K. Adesehinwa, Utilisation of palm kernel cake as a replacement for maize in diets growing pigs: Effects on performance, serum metabolites, nutrient digestibility and cost of feed conversion, Bulgarian Journal of Agricultural Science 13 (5) (2007) 593-600.

[2] A.E. Onyimonyi, S.O.C. Ugwu, Biological indices of broiler chicks fed various rations of cassava peel and bovine blood, International Journal of Poultry Science 6 (5) (2007) 318-321.

[3] K.U. Amaefule, S.N. Ibe, S.F. Abasiekong, O.C. Onwudike, Nutrient utilization of growing pigs fed diets of different proportion of palm kernel meal and brewer's dried grains, Pakistan Journal of Nutrition 8 (4) (2009) 361-367.

[4] A.O.K. Adesehinwa, B.K. Ogunmodede, I.I. Dafwang, T.S.B. Tegbe, A review of utilization of some agro-industrial by-product in pig rations, Nigerian Journal of Agricultural Extension 11 (I and II) (1998) 50-64.

[5] B.L. Fetuga, O.O. Tewe, Potentials of agro-industrial by-products and crop residues as livestock feed components, Nigeria Food Journal 2 (1975) 136-142.

[6] B.L. Fetuga, G.M. Babatunde, V.A. Oyenuga, The value of palm kernel meal in finishing diets for pigs: 1. The effect of varying the proportion of protein contribution from blood meal and palm kernel meal on the performance and carcass quality of finishing pigs, The Journal of Agricultural Science 88 (1977) 655-661.

[7] E.A. Iyayi, O. Ogunsola, R. Ijaya, Effect of three sources of fibre and period of feeding on the performance, carcass measure, organs relative weight and meat quality in broilers, International Journal of Poultry Science 4 (2005) 695-700.

[8] Association of Official Analytical Chemists, Official Methods of Analysis, 15th ed., Washington DC, 1995.

[9] Statistical Analysis Software, Release 8.1., SAS Institute Inc. Cary, North Carolina, USA, 2000.

[10] K.U. Amaefule, S.N. Ibe, S.F. Abasiekong, O.C. Onwudike, Response of wearner pigs to diets of different proportions and high levels of palm kernel meal and brewer's dried grains in the humid tropics, Pakistan

Journal of Nutrition 5 (2006a) 461-466.

[11] A.O. Aduku, Tropical Feedstuff Analysis Table, Department of Animal Science, Ahamadu Bello University, Zaria, Nigeria, 1993.

[12] D.Y. Kil, S.N. Ryu, L.G. Piao, C.S. Kong, S.J. Han, Y.Y. Kim, Effect of feeding cyanidin 3-gluoside (C3G), high black rice bran on nutrient digestibility, blood measurements, growth performance and pork quality of pigs, Asian-Australian Journal of Animal Science 19 (12) (2006) 1790-1798.

[13] D. Babcock, Rice bran as a source of dietary fibre, Cereals Foods World 32 (1987) 538-539.

[14] I.D.I. Yaakugh, T.S.B. Tegbe, Performance and carcass characteristics of grower and finisher pigs fed diets containing brewers' dried grain, Nigerian Agricultural Journal 24 (1990) 31-40.

[15] J.P. Alawa, N.N. Umunna, Alternative feed formulation in the developing countries: Prospects for utilization of agro-industrial by-products, Journal of Animal Research 13 (1993) 63-98.

[16] G.M. Babatunde, B.L. Fetuga, V.A. Oyenuga, A. Ayoade, The effect of graded levels of brewers' dried grain and maize cobs in diets of pigs on their performance characteristics and carcass quality, Nigerian Journal of Animal Production 2 (1975) 119-133.

[17] G. Simpson, Comparative feed value for swine (factsheet), Ontario Ministry of Food, Agriculture and Rural Affairs, 2012, Order No. 97-035, www.omafra.org/Comparative Feed Value for Swine.htm (accessed July 15, 2012).

[18] H. Jorgensen, X.Q. Zhao, B.O. Eggum, The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hindgut and metabolism of pigs, British Journal of Nutrition 75 (1996) 365-374.

Journal of Agricultural Science and Technology A 4 (2014) 155-168
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Livestock Feed Marketing in Ethiopia: Challenges and Opportunities for Livestock Development

Mesfin Dejene[1, 2], Seyoum Bediye[2], Dawit Alemu[3], Getu Kitaw[2], Aemiro Kehaliw[2], Getnet Assefa[3] and Getaw Tadesse[4]

1. QAAFI, Centre for Animal Science (CAS), the University of Queensland, Gatton QLD 4343, Australia

2. Ethiopian Institute of Agricultural Research, Holetta Research Center, Addis Ababa, Ethiopia

3. Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia

4. International Food Policy Research Institute (IFPRI), Addis Ababa, Ethiopia

Received: December 23, 2013 / Published: February 20, 2014.

Abstract: This paper presents the feed marketing systems of Ethiopia in terms of feed demand and supply, feed quality issues, feed prices and price trends based on qualitative data generated through rapid market appraisal methodology. The results indicate that, the demand for roughages, agro-industrial by-products (AIBP) and compound feeds is showing increasing trend. The use of feed from commercial sources is, however, very limited due to shortage of feed supply and inefficient marketing system. The AIBP mainly from flour and grind mills, oil processing plants and breweries are in short supply and directly marketed to user or through traders. Consequently, most of the exiting feed mixers/processing enterprises are operating under capacity estimated at about 20%-30%. The main marketed roughages, which are mainly cereal straws and baled hay, are also in short supply. Feed prices are increasing from time to time and mainly exacerbated by the increasing trend in export market of AIBP and double taxation in mixed rations. There is no any feed quality control or assurance mechanism in Ethiopia. Precaution needs to be taken in the area of taxation in order to avoid double taxation. Implications to improve the feed marketing systems and opportunities for livestock development are drawn.

Key words: Agro-industries, by-products, concentrate mixtures, crop residues, quality control, taxation.

1. Introduction

Subsistence livestock production constitutes a very important component of the country's agricultural economy accounting for 16% of the total GDP and over 40% of the agricultural GDP [1], 15% of export earnings and generates 30% of the agricultural employment [2]. Moreover, livestock are estimated to contribute to the livelihoods of 60%-70% of the Ethiopian population. More interestingly, the livelihood of pastoralists is dependent on livestock. Pastoral areas cover 60% of Ethiopia and include 12%-15% of the human population, as well as very large number of livestock [3]. The major contribution of livestock to food security and poverty reduction is expected to increase substantially because of increases in standard of life and urbanization in developing countries.

Despite its contribution to the economy and small holders' livelihood, the production system is not adequately market-oriented and livestock productivity remains very low due to various constraints that include poor nutrition and disease prevalence. These problems are compounded by inefficiencies in the input (feed, genetic material and veterinary services) and output (livestock and livestock products) marketing, including poor market infrastructure, lack of marketing support services and limited market information [4]. Among these constraints issues related to feed are the most remarkable ones. Feed shortage in quantity and quality has been a critical problem in Ethiopian livestock

Corresponding author: Mesfin Dejene, Ph.D. candidate, research field: agriculture (animal nutrition). E-mail: mesfin.ejigu@uq.net.au; mesfindegene@yahoo.co.uk.

production system [4-6]. The single largest expense in animal production is feed cost and it dictates feasibility of livestock enterprise. Overall, among the dominant factors contributing to the feed shortage both in terms of quantity and quality is the poor feed marketing system characterized by poor market information, localized thin markets and limited premium price for quality.

A review of past research works indicated that animal feeds and nutrition research largely concentrated on biological aspects both on-station and on-farm. If adoptions of animal nutrition technologies have to be optimized on the on-farm, feed technology development should be accompanied by efficient feed market system. An efficient feed marketing system is rewarding both for marketing agents and livestock producers. So far, very few studies have addressed issues of feed supply and marketing [4]. However, information concerning livestock feed demand and supply, feed quality issues, feed marketing, feed prices, price trends are scarce. This paper is aimed at assessing the feed marketing systems in Ethiopia in general and in the Central Ethiopia in particular, to analyze and generate a qualitative understanding of the feed demand and supply situations, feed quality issues, feed marketing, feed prices and price trends.

This paper is organized as follows: The next section presents the methodology used in the study. Section three presents results and discussion on domestic livestock feed supply and demand, export demand of livestock feeds, domestic livestock feed marketing and prices, feed quality and government policy issues. Section four concludes the paper and presents implications.

2. Materials and Methods

This study was conducted based on Rapid Market Appraisal (RMA) methodology [7, 8]. As key marketing reference points different markets in North Shewa (Sululta, Chancho, Fitche and Gerba Guracha), West Shewa (Holetta, Addis Alem, Ginchi and Ambo), South West Shewa (Alemgena, Sebeta, Tulubolo and Weliso), East Shewa (Dukem, Bishoftu, Mojo and Adama) and Addis Ababa and surroundings were selected as main sites of investigation and sources of information of marketing operations.

The study is based on qualitative information such as feed supply and demand aspects, feed quality issues, challenges and opportunities of feed marketing sourced through group discussions and interviews with key informants (hay producers, millers and feed processors, small and large scale agro-industries, feed traders, feed buyers and cattle traders). Data were also collected based on interviews with key informants (experts of the respective Woreda Offices of Agriculture and Rural Development and Development Agents, experts of the Respective Woreda/Urban Offices of Trade, industry and transport, experts at the Ministry of Agriculture and Rural Development (MoARD), Ministry of Trade and Industry, Bureau of Trade & Industry (Addis Ababa), Zonal Offices of Agriculture and Rural Development and experts of the Urban Agriculture Offices).

In addition to this, data generated through personal observations through field visits (feed market places, livestock farms, small and large scale agro-industries (edible oil factories, floor factories and millers) and feed processing industries) were considered. Data on feed prices and price trends, type and amount of feed exported were collected from secondary sources (records of federal, zonal and district level, Ethiopian Customs Authority, Addis Ababa Chamber of Commerce, traders, small and large-scale agro-industries and feed millers and mixers/processing enterprises).

3. Results and Discussion

3.1 Domestic Livestock Feed Supply

3.1.1 Types of Livestock Feed Supplied in Domestic Market

In general, the feed markets can be categorized into three main market types: markets for roughages, markets for agro-industrial by-products (AIBP) and

markets for compound feeds/concentrate mixtures/formulated rations.

3.1.1.1 Roughages (Crop Residues and Natural Pasture Hay)

The types of crop residues in the country differ from place to place depending on the type of crop grown as determined by the agro-climatic conditions. The major crop residues supplied in the market are cereal crop residues like teff straw, barley/wheat straw, green maize fodder, sorghum stover and oat (*Avena sativa*) fodder. However, there is a limited supply of pulse crop residues. The area of grazing land has declined markedly particularly in the highlands, and this trend is continuing at an increasing rate due to expansion of crop cultivation and urbanization, and to a lesser extent through land degradation [9]. Hence, the supply of natural pasture hay is diminishing while crop residues are becoming increasingly important in the annual feeding cycle, already accounting for more than 50% of total feed in most areas. Sugarcane tops and bagasse are mainly produced by the state sugar factories. However, because of its bulky nature and difficulty to transport, most of the sugar cane tops are either burned or left in the field and used freely by any livestock producers living in the vicinity of the factories.

3.1.1.2 AIBP

The major feed resources as by-products of the agro-industries in the country are milling by-products (wheat bran (the coarse outer coat of wheat), wheat middling (finer which may contain bran, endosperm and germ), wheat short, rice bran and screenings), edible oil processing by-products such as nougseed (*Guizotia abyssinica*) cake (NSC), cottonseed cake (CSC), linseed cake (LSC) and rapeseed (*Brassica carinta*) or Ethiopian mustard cake (RSC), groundnut, sesame, sunflower, peanut, safflower cakes, etc., molasses and spent brewery grain. The traditional brewery residue (*Tela atella*) and/or traditional liquor residue (*Katicala/Areqe atella*) are also the by-products produced by small scale brewery and liquor plants, respectively. The major producers of wheat by-products are flour mills. Wheat bran is the most common by-product marketed and used for livestock feeding. According to Tolera [6], there are about nine state and 181 private owned grains milling factories in the country with operating capacity of 73% and 55%, respectively, mainly producing wheat by-products (Table 1).

Oil crop by-products are produced mainly by large (Table 2) and small scale edible oil processing factories. The types and the importance of the particular seed cakes in the country vary from place to place. In west, South West and North Shewa zones, the seeds cakes traded are noug and linseed cakes. In Adama town, cottonseed, linseed and nougseed cakes are most supplied.

Most of the large-scale edible oil factories are operating at less than 50% of their capacity due to fluctuation and inadequate supply of raw materials (oilseeds) because of competition with export and direct use of the seeds locally, high price of oilseeds, availability of cheaper imported oil in the local market and climatic factors.

According to administrative zone/town/district trade, industry and transport office and/or district finance office and bureau of trade and industry (Addis Ababa), the number of small scale oil processing plants

Table 1 Total quantity sold of wheat bran (WB) and wheat middling (WM) from private and state owned food industries used as animal feed, during 2003/04-2006/07.

Name of agro-industry	Type of by-product	Total quantity (quintals) sold			
		2003/04	2004/05	2005/06	2006/07
DH-GEDA Floor Factory	WB	NA	19,798.5	15,356.5	15,295.5
Kaliti Food Share Company	WB	23,829.7	25,374.2	14,591.4	13,969.5
	WM	16,513.0	15,441.7	9,522.4	11089.8

Source: DH-GEDA Floor Factory (AA) and Kaliti Food Share Company; NA: not available.

Table 2 Annual production* (total quantity sold) of different oilseed cakes used as animal feed, during 2003/04-2006/07.

Name of agro-industry	Type of by-product	Total quantity (quintals) sold			
		2003/04	2004/05	2005/06	2006/07
Addis-Modjo Edible Oil Complex Share Company	CSC/Meal	NA	11,588.86	10,486.40	14,395.00
	NSC/Meal	NA	NA	3,702.29	364.50
	RSC/Meal	NA	5,102.41	13,280.19	15,633.82
Total			16,691.27	27,468.88	30,393.32
Nazareth Edible Oil Complex Share Company (Adama)	CSC	NA	21,364.90	25,044.20	35,204.30
Hamaressa edible oil (Harar)*	Peanut cake	3,470	2,090	2,380	NA
Bahir Dar edible oil (Bahir Dar)*	Oilseed cake	2,290	1,110	2,660	NA

Source: large scale edible oil factories and *compiled from Ref. [6]; NA: not available.

available in nine districts of West Shewa, six districts of North Shewa and two districts of South West Shewa zone, and in Addis Ababa, Bishoftu and Adama are 42, 16, 4, 46, 2 and 39, respectively. According to the small-scale edible oil processing plant owners at Kuyu District (North Shewa zone), depending upon the efficiency, an oil processing plant can process up to 4 quintals of noug seed and produce 96-100 L of oil and 2 quintals of NSC per day. However, one should note that it was not easy to get and/or estimate information on the amount of oilseed cakes produced and the level of production performance of their plant by most privately owned small-scale oil processing plants located in various parts of the country, presumably for concerns related with taxes.

Molasses is mainly produced by the state sugar factories namely Wonji-Shewa, Metehara and Finchaa sugar factories (Table 3). It is the cheap source of soluble carbohydrate for livestock, and is highly palatable and used for flavor and control of dust in ration. However, because of the competing alternative use (ethanol production), increasing trend in export market and its bulky nature and difficulty of transport, the amount of molasses used as animal feed is quite insignificant and is not properly marketed in the country. Currently, the molasses produced from Finchaa Sugar Factory is used for ethanol production [6].

Spent brewery grain is produced from Meta, St. Goerge, Bedele, Harar and Dashen brewery factories. It is an excellent source of protein and a good source of energy, and is highly palatable and can be used in a variety of rations. In 2006/07, annual production of St. George brewery, Bedele brewery and Harar brewery were 1,200 MT, 88,000 HL and 53,365 MT spent grain, respectively. In the same year, Bedele brewery also produced 20,000 HL brewer's yeast in addition to spent grain while Awash Winery produced 100 MT of by-product [6]. Breweries offer this by-product only in the wet form (70%-80% moisture), therefore transportation costs are high. Unless stored in trench or bunker silos, the shelf life of brewer's grain is limited to 3-4 d, depending on the environmental temperature. The storage and cost of handling usually also limit the use of brewer's rain. Consequently, some breweries like Meta Abo Brewery Share Company (MABSC) sell spent grain as by-product only around their production areas (Table 4).

The majority of food industry surveyed did not have a strategic plan to handle their by-products that can be utilized for animal production. Among the by-products that are not properly marketed and can be used for ruminant production are: brewers grains, brewers yeast, tomato pomace, grape pomace, sugarcane tops, bagasse, pasta waste, wheat short and wheat screenings. The use of molasses is also very limited due to supply shortage mainly associated with domestic marketing problem and this problem becomes more serious in distant places from its production areas.

Table 3 Annual productions of molasses from sugar factories, during 2003/04-2006/07.

Name of agro-industry	Type of by-product	Total Quantity (MT)			
		2003/04	2004/05	2005/06	2006/07
Metehara	Molasses	35,749	37,727	41,343	41,455
Wonji-Shewa	Molasses	18,754	18,386	17,901	17,579
Finch'a	Molasses	NA	22,225	21,607	24,376

Source: Ethiopian Sugar Development Agency; Ref. [6]; NA: not available.

Table 4 Total quantity sold (in dry matter basis) of brewers spent grain from MABSC, during 2004/05-2009/10.

Name of agro-industry	Type of by-product	Total quantity sold in MT				
		2004/05	2005/06	2006/07	2008/09	2009/10
MABSC	Spent grain	2,138.40	2,317.70	2,981.90	1,923.50	1,899.70

Source: Meta Abo Brewery Share Company (MABSC), Sebeta.

Dairy producers at Kuyu District reported that the supply of AIBP such as wheat bran, oilseed cakes and molasses are lower than the demand at prevailing price, and most of the time they are unavailable. However, it should be mentioned that in most of the potential peri-urban and urban areas of the country, there is availability problem. The status of feed supply in the country is at an alarming state where the supply shortage problem is not limited to only the shortage of own produced feed or naturally available feed such as from grazing lands, but also the unavailability of feed supply for those who can afford to buy [10]. This has a direct implication in the promotion of livestock production especially in urban and peri-urban areas where there is huge investment in livestock production (dairying, fattening and poultry).

3.1.1.3 Compound Feeds (Formulated Rations)

Among the 15 feed mixers and millers found in the country [4], eight of them are found along the Addis Ababa Adama corridor engaged in compound feed production using purchased grains, AIBP and imported vitamins and minerals. Usually, these enterprises secure the purchase of inputs through participation in auctions of flour mills. They sell the compound feeds directly to users; no traders are involved in the sell of compound feeds. The current market channels for compound feeds are characterized by (1) the direct sale to individual purchasers (livestock owners including equines), who normally buy small quantities ranging from 1 to 5 quintals per purchase; (2) participation in auctions to deliver to intensive private as well as government livestock farms and for relief purposes; (3) supplying compound feed based on orders which is commonly practiced by NGOs that promote livestock production.

Most feed mills complain about unavailability and price rise of raw materials (AIBP), fluctuation of raw materials supply both in quantity and quality and price with season. Better quality raw material is available at crop harvest and vise-versa. Recently, they are facing a lack of raw material such as soybean in the local market used to formulate poultry ration. Former private big clients of some of these feed mills decided to run their own feed mixing operations, competing in the procurement of raw materials. Experts' estimates suggest that only about 25% of the AIBP are converted into compound feeds that are formulated to give the required nutritional composition for the different types of animals and the rest 75% is used directly to feed animals. Consequently, most of the exiting feed processing enterprises are operating under capacity estimated at about 20%-30%. Moreover, they are not technically updated. Equipment used is old and rudimentary. Lack of skilled man power, modern facilities and technology also has an impact on the efficiency on the enterprises. Feed millers are not regulated in terms of feed tag quality or compliance. They can mix anything and do not have to include a

tag. Feed millers and mixers do not have advanced technology for storage, grinding, mixing, mix formulation or quality control laboratories. Lack of premium price for quality products due to lack of quality standards is also limiting their supply. The use of food-by-products by feed millers is limited to the most traditional by-products such as wheat bran, CSC or meal and NSC and a limited extent RSC. For ruminant diets they do not include trace minerals or vitamin supplements.

3.2 Domestic Livestock Feed Demand

3.2.1 Demand for Roughages (Crop Residues and Natural Pasture Hay)

In recent years, the market for roughages is booming due to the continuous reduction of grazing area and also expansion of commercial farms mainly in urban and peri-urban areas. The main roughages marketed are cereal straws and baled hay. The trade for baled hay is mainly dominated by commercial livestock farms, which follow two types of marketing practices: the first is a marketing practice where commercial livestock farms contact in pasture lands, harvest and bale and use for their own farm and sell the surplus to other livestock farms; the second practice is the purchase of dry hay by commercial farms from small-scale farmers who produce hay on small-scale bases on pasture lands. There is an increasing trend in market for straws and stover, particularly in areas adjacent to dairy and fattening programs. Recently, baled crop residues such as teff and barley/wheat straw are traded in some urban areas (Addis Ababa and Adama). However, the alternate use of crop residues such as teff straw for house construction and teff, barley and wheat straws for mattress making has made much contribution for the scarcity of the roughages for livestock feed. Basically the roughage feed market is informal.

3.2.2 Demand for AIBP

Oilseed cakes are used as a protein supplement whereas the by-products from floor mills used as an energy supplement to low quality crop residues and hays. AIBP principally bran, molasses and oil seed cakes have greatly increased in value. They are primarily used within fattening and dairying programs [9]. In general, the demand of the AIBP is lower during the wet season (July to September) due to the green fodder availability and the low fattening activities during the season. The AIBP are directly marketed to user or through traders who buy in large quantities for the purpose of retailing and making available in small quantities as animal feed. The customers are mainly small-scale dairy and fattening farms in urban and peri-urban areas as well as urban equine owners (horse cart and donkey owners), where the agro-industries are found. For instance, dairy producers in urban areas of the southern part of the country (Hawassa, Yirgalem and Dilla) use mainly purchased roughage and AIBP along with non-conventional feeds like “attella” and also hay stacking especially during dry period [11]. However, the marketing of brewery by-products both from commercial breweries (brewery grain) and home making breweries (Tella Atella) and liquor (Areke Atella) is limited only around the production areas due to the transport problem associated with the bulky nature of these by-products.

3.2.3 Demand for Compound Feeds

The markets for the different compound feeds are concentrated along the stated corridor, where the feed processing enterprises and intensive and semi-intensive livestock farms (dairy, cattle fattening, poultry and pig) are found. Feed millers complain that private livestock producers do not use their products for the following reasons: high price per quintal, lack of trust on their product (lack of animal performance), lack of awareness/knowledge about the existence of complete manufactured animal feeds and the majority of livestock producers in the country are subsistence and not market-oriented. They stated that the price rise of compound feeds is associated with the general increase in food prices coupled with lack of tax

exemptions on raw materials (AIBP) and double taxation on their products. Consequently, most of the urban and peri-urban livestock producers decided to run their own feed mixing operations and resulted in diminishing of compound feed market. However, they do not make efforts to create their markets through better services and technical assistance or advertising. On the other hand, low purchasing power of the smallholder/subsistence farmers due to lack of market oriented livestock production system resulted in a great impact on the potential market of compound feeds. The total demand of compound animal feed is low. Furthermore, there is low demand of compound feeds during fasting time. As a result, the current low demand for compound feeds have either gave incentive for most of the feed processing enterprises to practice vertical integration, i.e., engagement in modern livestock production (fattening, dairy, poultry and/or pig farming), or they are forced to sale their respective products either with minimum profit margin or sale a product having inferior quality at low price.

3.3 Export Demand of Livestock Feeds

The export data from the year 1997-2010 indicates that among the exported livestock feed types, in terms of volume and free on board (FOB) value, the highest export market share goes to molasses (63.57% and 39.75%) followed by crop residues/hay (18.76% and 29.59%), oil seed cakes (12.80% and 25.05%), brans (4.867% and 5.59%) and compound feeds (0.003% and 0.015%), respectively (Fig. 1). All types of feed are mostly exported to Djibouti and Arabian Peninsulas. Recently in 2009 and 2010, a significant amount of oil seed cakes mainly RSC are exported to China followed by Sudan, Vietnam and Singapore while brans are exported to Somalia. In all types of feeds except compound feeds the volume of export market is increasing since 1997. Even though there is export ban of animal feeds in this country since 2009, there is still an increasing trend in export market of oilseed cakes (RSC) followed by brans, crop residues/hay and molasses from January to June, 2010 (Fig. 1).

AIBP are inputs for feed processing enterprises. However, a large volume of these feed ingredients are being exported without any value addition and resulted in a supply shortage for domestic market. The trend in the FOB value (in Ethiopian Birr) of total feed exported from Ethiopia shows that there is huge increase in 2004, 2007 and 2010 as compared to the previous years even though there was a fluctuation from year to year since 1997 (Fig. 2).

3.4 Domestic Livestock Feed Marketing and Prices

The feed market types and marketing practices are

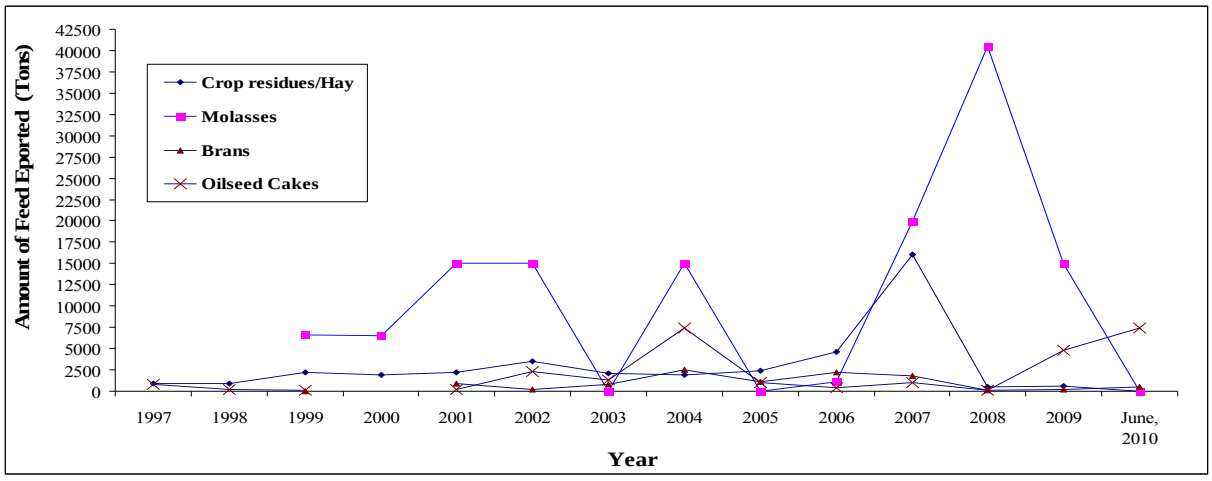


Fig. 1 Amount of animal feeds (t) exported during 1997-June, 2010.

Source: Ethiopia Custom Authority and Addis Ababa Chamber of Commerce.

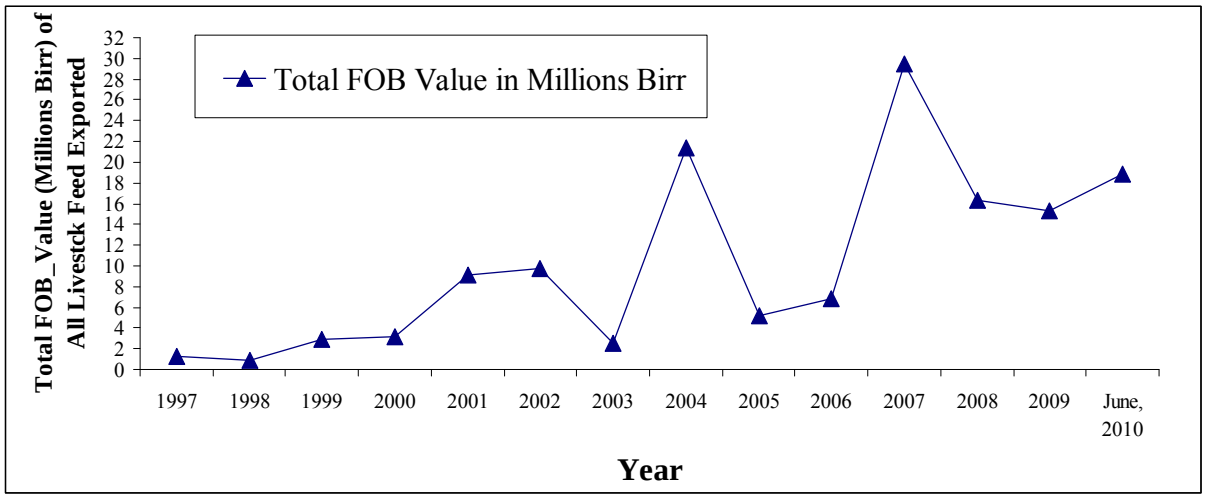


Fig. 2 Trends in the FOB value (Birr) of total feed exported from Ethiopia since 1997-June, 2010.
Source: Ethiopia Custom Authority and Addis Ababa Chamber of Commerce.
1USD ≈ 9.9 Birr.

different depending upon the livestock production system along with the types of feed. Similarly, the markets for urban, peri-urban and rural areas vary considerably. In urban and peri-urban areas where there is intensive and semi-intensive livestock production in the form of dairying, ruminant fattening, poultry and pig, relatively, there is considerable market for compound feeds, AIBP and also for roughages mainly for baled hay and straw. In rural areas where there is a mixed crop-livestock production system, majority of the livestock producers use feeds from their own farms. For instance, about 54% of dairy producers in southern part of the country use solely feeds from their own farms and about 24% use both feed from their own farms and communal grazing [11]. On the other hand, in the pastoral production system, the source of feed is mainly free grazing without as such market for any type of feed.

3.4.1 Price and Price Trends of Roughages (Crop Residues/Hay)

Following the general price increase in the economy, roughage prices have also risen sharply in recent years. In general, roughage prices tend to be higher during the dry and wet seasons and lower during harvesting season. Similarly, there is considerable variability in prices among different areas in the country, which is reflected in the price ranges for the different types of straw (Table 5). In 2009/10 the price of teff straw ranges from 0.8 Birr/kg to 2.5 Birr/kg, which is mainly the reflection of the multipurpose use of teff straw as animal feed also as input for construction. The price of baled hay ranges from 0.7 Birr/kg to 2.0 Birr/kg, which is the reflection of location variability. Moreover, the price of hay varies with the quality. The higher the quality, the better will be its price.

3.4.2 Price and Price Trends of AIBP

The prices of the different by-products have been consistently rising from time to time. Though the highest price increment of the AIBP was observed in 2007/08, the price for NSC has increased from 54 Birr/quintal in 2003/04 to 185 Birr/quintals in 2009/10. Similarly, the price of wheat bran has increased from 52 Birr/quintal in 2003/04 to 140 Birr/quintal in 2009/10 (Fig. 3). The price of all AIBP showed a slight decrease in price during 2008/09 due to the government intervention in importing wheat grain and exemption of tax in consumable products. However, the price of AIBP is still rising in 2009/10. The increasing price trend is associated with the overall price increase in agricultural products and also increased demand for these by-products following the expansion of commercial livestock farming mainly in urban and peri-urban areas.

Table 5 Price ranges for different roughage feeds in 2008 and 2009/10.

Feed type	Price range ($Birr/kg), 2008*	Price range ($Birr/kg), 2009/10#
Teff (*Eragrostis tef*) straw	0.50-1.70	0.8-2.5
Barley/wheat straw	0.25-1.00	0.4-1.2
Sorghum stover	0.20-0.65	-
Loose natural pasture hay	0.30-1.00	0.5-1.4
Baled natural pasture hay	0.50-1.66	0.7-2.0
Green oat (*Avena sativa*) fodder (N. Shewa)	0.50-0.80	0.8-1.40

Source: *Ref. [4]; #field survey result in towns (open and farm gate markets).
1USD ≈ 9.9 Birr.

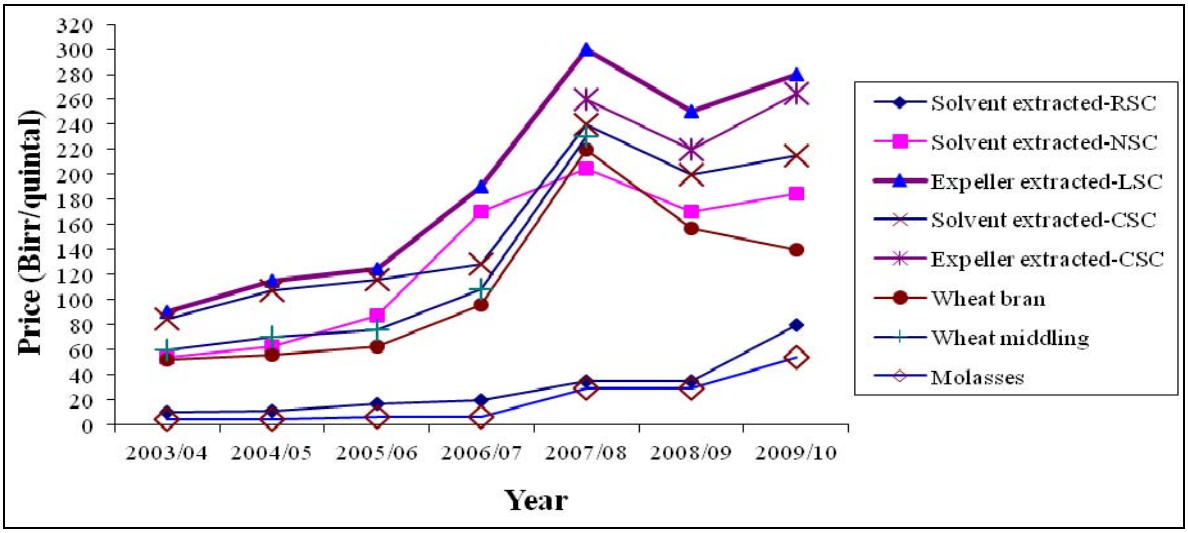


Fig. 3 Trends in the prices of AIBP (Birr/quintal) during 2003/04 to 2009/10.
Source: Addis-Mojo Edible Oil Complex Share Company (AMEOSC), Nazareth Edible Oil Factory, Ethiopian Feed Industry Association, Ethiopian Sugar Development Agency (Addis Ababa) and Farmers.
1USD ≈ 9.9 Birr.

Among the oil seed cakes, LSC is the most expensive followed by CSC and in contrary RSC is the cheapest of all; this is due to its less demand as a result of its high anti-nutritional (glucosinolates (GSLs)) contents. The use of RSC/meal within compound feeds is also usually restricted by the presence of these endogenous anti-nutritional factors or anti-nutrients.

GSLs are known for a long time to reduce the intake, induce iodine deficiency, hypertrophy of liver, kidney and thyroid and at higher levels mortality. Deleterious effects of GLSs are greater in non-ruminant animals compared to ruminants [12]. In 2009/10 the price record of AMEOSC indicates that expeller extracted CSC is more expensive (265 Birr/quintal) compared to the price of solvent extracted CSC (215 Birr/quintal). Moreover, in 2007/08 expeller extracted RSC is also expensive (160 Birr/quintal) than the price of solvent extracted RSC (35 Birr/quintal) which is in agreement with Ref. [12] indicated that oil extraction process affects the total GSL (μmol/g) content of the meals because of varying oil extraction conditions. Solvent-extracted meals contain higher amount of GLS than that in dehulled extracted meals. Expeller extracted rape seed meal contains less GLS content than in solvent extracted meal.

Along with other by-products, the price of molasses is also increasing from time to time. The factory price of molasses has increased from 4.56 Birr/quintal in

2003/04 to 54.05 Birr/quintal in 2009/10. However, the trend for molasses is a bit different from the rest of AIBP in that it did not show a decrease in price during 2008/09. This may imply that molasses price is triggered by some other factors rather than the general food price increase in the country. As indicated in Fig. 1, molasses is one of the highly exported animal feeds during 2007 and 2008. Taking the use of molasses as a feedstock for production of bio-fuel into consideration, the demand is expected to increase and will result even in more price increase. However, the factory gate price (in dry matter basis) of spent grain at Meta Abo Brewery Share Company remains the same (5.6 Birr/quintal) for the year 2004/05 to 2006/07 with a slight increase (6.4 Birr/quintal) in 2008/09 and 2009/10; this might be due to its limited utilization only around production areas associated with its bulky nature, high transportation cost and limited shelf life.

The average (2004/05 to 2009/10) monthly price of wheat bran has shown seasonality in a year where the price increases in the months from March to June as compared to other months (Fig. 4). This trend is mainly due to higher demand in the early months of the rainy season.

3.4.3 Price and Price Trends of Compound Feeds (Formulated Rations)

The trend in the prices shows that there is huge increase in 2007/08 as compared to the previous years even though there was a gradual increase from year to year since 2003/04 (Fig. 5) due to the increasingly supply shortage and price hike of the inputs such as AIBP.

Price set of compound feeds is determined by cost of production plus profit and value added tax. The seven years (2003/04 to 2009/10) average price data show that poultry rations (starter ration followed by layers and growers ration) have the highest price/quintal followed by dairy, calves, sheep, heifers, beef and bull ration (Fig. 6). This is due the difference in input price of the ingredients used for formulation of ration. Vitamin premix and lysine are imported ingredients used for poultry ration and this makes poultry ration expensive compared to other livestock ration.

In general, prices of most feed types are increasing over time. The reasons of such price change are related to changes in domestic and demand, domestic supply shortage and policy related issues.

3.4.4 Effects of Feed Price Rise

Due to the continuous rise in feed prices relative to the price of milk and milk products, most of urban and per-urban dairy farmers have either obliged to destock their dairy cows or decided to maintain their animals by underfeeding for some period of time which resulted in a sharp reduction in animal productivity and would affect the lifetime productivity of dairy cows. Moreover, most of the feed processing industries were operating far below their potential

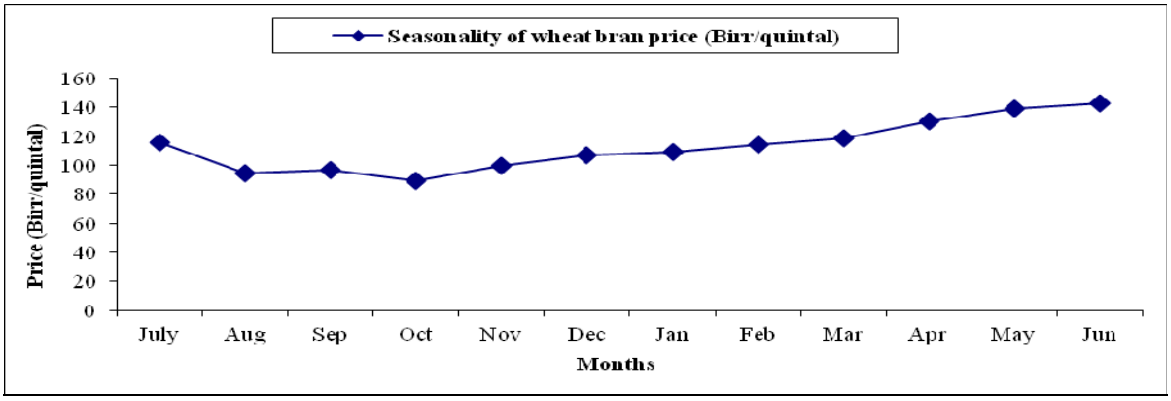


Fig. 4 Seasonality of wheat bran price (factory gate) at Addis Ababa during 2004/05 to 2009/10.
Source: DH-GEDA Floor Factory, Addis Ababa.
1USD ≈ 9.9 Birr.

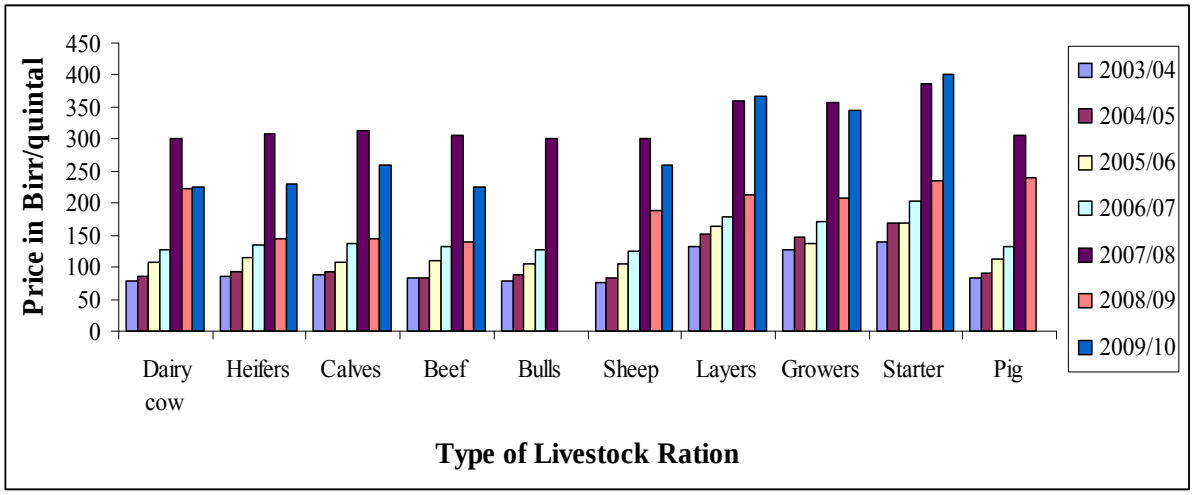


Fig. 5 Trends in prices of compound feeds for different classes and types of livestock (2003/04-2009/10).
Source: Kaliti Feed Processing Enterprise and Ethiopian Feed Industry Association.
1USD ≈ 9.9 Birr.

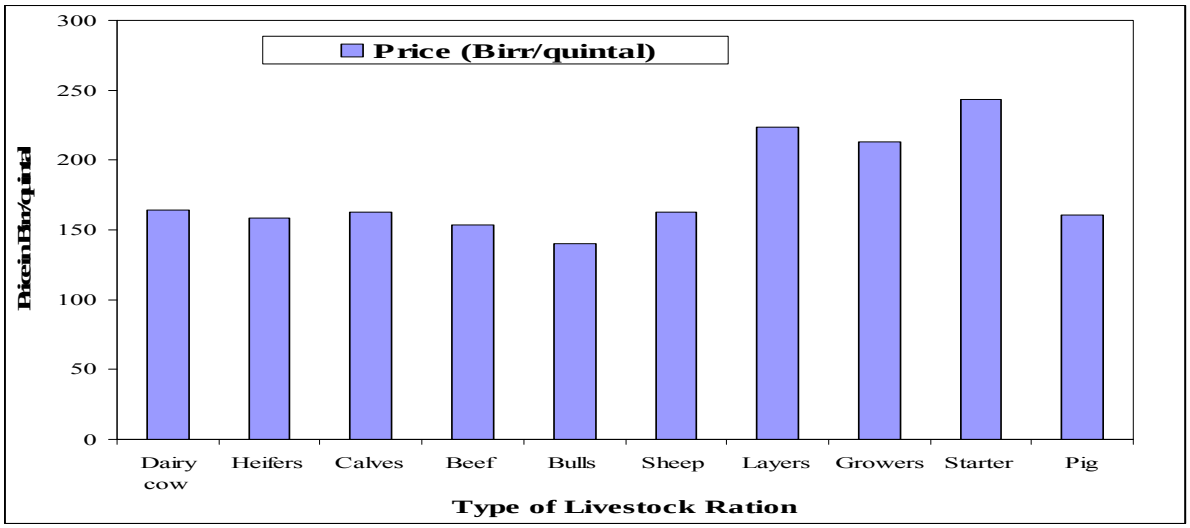


Fig. 6 Average prices of compound feeds for different classes and types of livestock from the year 2003/04 to 2009/10.
Source: Kaliti Feed Processing Enterprise and Ethiopian Feed Industry Association.
1USD ≈ 9.9 Birr.

which might lead them on the verge of collapse due to the continuous input price rise coupled with unavailability of raw materials at prevailing price and low demand for compound feeds. This has to be reversed in order to make these industries inline with the current government development and transformation plan.

3.5 Feed Quality Issues

The feed quality issue is related with fulfillment of the nutritional requirements of the livestock under consideration with minimum possible cost. Therefore, the quality of feed considers the nutrient requirement of the livestock under consideration and the nutritive value of feed ingredients available. Determination of these parameters avoids under or overfeeding of nutrients. Underfeeding can cause impaired performance of animals whereas over feeding would increase feed cost. Cognizant of the need to ensure feed quality, different manuals were prepared for practitioners [13-15].

Even though, the market for the different feed type

is increasing from time to time, there is not any feed quality control and assurance mechanism in Ethiopia [6]. Most of the dairy, fattening and poultry farms use their own feed formulation due to the limited number of feed processing companies, lacking of awareness and the increasing trend in prices of compound feeds. Moreover, some livestock farms that buy compound feed complain about the quality mainly related with the poor nutritional composition. This has created mistrust among actors in the industry further thinning the compound feed market, which needs to be addressed through implementation of vibrant public quality and standard enforcement mechanism.

3.6 Government Policy Issues

The ineffectiveness of the export ban and excessive value added tax are the two most important policy issues that trigger the persistence of high animal feed prices. Following the increasing trend in feed prices over the years, recently Ethiopian feed industry association was established with the objective to solve the problem associated with markets for compound feeds. Moreover, the association requested the government to intervene on the increasing trend of feed export market especially on non-value added feed types such as AIBP and crop residues/hay and on the taxation. Consequently, the government has banned feed export market. However, our study indicates that still there is feed export market. This might be associated with lack of equal play field in the export market, transparency and awareness among all the stakeholders who are implementing the policy. Moreover, we have observed that while value added tax is exempted for most food crops, AIBP (inputs for feed processing enterprises) were not exempted and consequently there is also double taxation in formulated (compound) feeds. So long as the objective of tax exemption is to reduce the consumers food cost, animal feed would have been exempted since feed cost usually accounts for 60%-70% of the total livestock production costs. Such double taxation coupled with unregulated export market of AIBP has inflated compound feed prices in an unprecedented rate.

4. Conclusions

The market transactions for roughages, AIBP and compound feeds is showing increasing trend, even though its size is still small considering the livestock population the country. Crop residues such as teff straw, barley and wheat straw and natural pasture hay are the most marketable roughage feed in the study area and used as a basal diet for livestock. Natural pasture areas are declining overtime due to the expansion of crop cultivation and urbanization. Crop residues and natural pasture hay are also exported to Djibouti and Arabian Peninsula. Hence, in most of the study areas there is an acute shortage of natural pasture hay supply in domestic market at prevailing prices.

The most commonly marketed AIBP in the study area are noug, linseed, cotton seed cakes, wheat bran and to limited extent molasses and RSC. The export market of AIPB has made a contribution for the shortage of AIBP and increased their prices in the domestic market. The AIBP which are used as raw material for compound feeds are not tax exempted and there is also double taxation in the formulated/compound ration. As a result, prices of compound feeds are also rising sharply.

In general, commercial feed supply is emerging in urban and peri-urban parts of the study areas. The use of feed from commercial sources is, however, very limited due to shortage of feed supply and inefficient marketing system and this problem become more serious in rural areas. In most of the potential peri-urban and urban areas of the country there is feed availability problem. Moreover, there is a variety of by-products that are available for livestock feeding, but not all properly marketed and utilized. This may be due, in part, to ignorance about availability and/or suitability for use in animal nutrition. Therefore, there

is an opportunity to use these by-products in a national way in order to increase profitability, and improve animal nutrition and performance. Hence, there is a need for interventions to develop feed markets in Ethiopia. Based upon this, we draw the following implications.

In order to promote livestock production, the feed production and marketing aspect need to get due attention. Interventions to increase the feed supply, improving ration formulations both in the nutritional and economic area and developing feeding strategy based on locally available feed resources are urgently needed which could vary depending on the resource base of a particular intervention area. Moreover, ways should be devised for the better conservation, utilization and marketing of underutilized alternative (non-conventional) feed resources produced as by-products from cannery, vegetable, fruit wastes, tomato pomace and grape wastes, sugarcane tops and bagasse from sugar factories, brewers grains and brewers yeasts from brewery factories etc..

Livestock production in Ethiopia should be commercialized (market-oriented) and feed marketing aspects have to receive at most focus and attention. In order to achieve commercialized livestock production system, an enabling environment including strategic policy in livestock feed marketing is the most important aspect that needs to be addressed and implemented in a value-chain approach. On the other hand, unless there is a wider investment in the sector mainly in modern/commercialized livestock farming (dairy, fattening, poultry etc.), the demand for compound feed will remain low. Thus, it is important that there is a planned promotion of modern livestock farming where the existing resources mainly the feed resource are utilized efficiently. On top of this, there is also an opportunity for private sectors to enter into the feed mill industry if the market can be created and expanded. Hence, it is also important to promote the private sectors to enter into the feed mill industry so as to supply affordable feeds for modern livestock farming as well as to the smallholder semi-commercialized livestock keepers. Therefore, (1) the public support in terms of agricultural extension, credit and infrastructures needs to be strengthened to support the sector mainly in urban and peri-urban areas in order to well link the feed sector with modern livestock production; (2) in order to increase demand for compound feeds for improved efficiency of the existing feed processing industries, different marketing strategies should be promoted like mini package of compound feeds, demonstration of the use of compound feeds to users, awareness creation through advertisement and training of livestock producers, and implementation of vibrant public quality and standard enforcement mechanism, linking cooperatives with feed supply, etc.; (3) investment promotion in animal feeds need to be promoted for increased competition in the feed markets; (4) there should be strict ban of export market on non-value added feed ingredients while up-grading the capacity of feed processing industries to compete in domestic as well as in international markets; (5) improving the transparency of market operations including government policies by providing equal access to all participants; (6) the cost items related with value added tax indicate a need for targeted policy and development intervention since they have big impact on the competitiveness of compound feeds in domestic as well as export market through inflated domestic cost of production. Thus, it needs critical policy decision to avoid double taxation.

The quantitative analysis of the existing livestock feed market (Domestic vs. Export) in a holistic system approach warrants further research, because feed cost is the major factor that dictates the feasibility of livestock production in Ethiopia.

References

[1] X. Diao, F. Belay, S. Haggblade, S.T. Alemayehu, W. Kassahun, Y. Bingxin, Agricultural growth linkage in Ethiopia: Estimates using fixed and flexible price models, International Food Policy Research Institute (IFPRI)

discussion paper, No. 00695, Development Strategy and Governance Division, Washington DC, 2007.

[2] S.J. Staal, A.P. Nin, J. Mohammad, Dairy development for the resource poor, Part 2: Kenya and Ethiopia dairy development case studies, Poor-Poor Livestock Policy Initiatives, PPLPI Working Paper, No. 44-2, 2008.

[3] M. Holdenman, The political economy of poor-poor livestock policy-making in Ethiopia: Poor-Poor livestock policy initiatives, PPLPI Working paper 19, 2004.

[4] B. Gebremedhin, A. Hirpa, K. Berhe, An appraisal of fodder marketing in Ethiopia, in: Proceedings of the 16th Annual Conference of the Ethiopian Society of Animal Production (ESAP) on Commercialization of Livestock Agriculture, Addis Ababa, Ethiopia, Part I, 2009, pp. 1-20.

[5] K. Hussen, A. Tegegne, M.Y. Kurtu, B. Gebremedhin, Traditional cow and camel milk production and marketing in agro-pastoral and mixed crop-livestock systems: The case of Mieso district, Oromiya Regional State, Ethiopia, Improving Productivity and Market Success (IPMS) of Ethiopian Farmers, Project working paper 13, ILRI (International Livestock Research Institute), Nairobi, Kenya, 2008, p. 56.

[6] A. Tolera, Livestock feed supply situation in Ethiopia, in: Proceedings of the 16th Annual Conference of the Ethiopian Society of Animal Production (ESAP) on Commercialization of Livestock Agriculture, Addis Ababa, Ethiopia, Part I, 2009, pp. 21-38.

[7] J. Holtzman, Rapid Appraisal of Commodity Sub-sectors: The Guide to Developing Agricultural Markets and Agro-enterprises, Abt Associates Inc., Bethesda, Maryland, USA, 1995.

[8] M. Morris, Rapid reconnaissance methods for diagnosis of sub-sector limitations: Maize in Paraguay, in: G. Scott (Ed.), Prices, Products and People: Analyzing Agricultural Markets in Developing Countries, Lynn Rienner Publisher, Boulder, USA, 1995.

[9] MoARD, Livestock Development Master Plan Study Phase I, Volume C—Forage and Seed Production, Ministry of Agriculture and Rural Development (MoARD) and GRM International BV, 2007.

[10] B. Gebremedhin, D. Hoekstra, S. Jemaneh, Heading towards commercialization?—The case of live animal marketing in Ethiopia, Improving Productivity and Market Success (IPMS) of Ethiopian Farmers, Project working paper 5, ILRI (International Livestock Research Institute), Nairobi, Kenya, 2007, p. 73.

[11] S. Yigrem, F. Beyene, A. Tegegne, B. Gebremedhin, Dairy production, processing and marketing systems of Shashemene-Dilla area, Southern Ethiopia, Improving Productivity and Market Success (IPMS) of Ethiopian Farmers, Project working paper 9, ILRI (International Livestock Research Institute), Nairobi, Kenya, 2008, p. 62.

[12] M.K. Tripati, A.S. Mishra, Glucosinolates in animal nutrition: A review, Animal Feed Science and Technology 132 (2007) 1-27.

[13] D. Fekadu, S. Bediye, Laboratory procedures for feed evaluation, Technical Manual 19, Ethiopian Institute of Agricultural Research (EIAR), 2007, p. 37.

[14] S. Bediye, Z. Sileshi, D. Fekadu, Chemical composition and nutritive values of Ethiopian feeds, Research report 73, Ethiopian Institute of Agricultural Research (EIAR), 2007, p. 24.

[15] A. Tolera, Feed resources and feeding management: A manual for feedlot operators and development workers, Ethiopia Sanitary and Phytosanitory Standards and Livestock and Meat Marketing Program (SPS-LMM) and Texas Agricultural Experiment Station (TAES), Texas A&M University, 2008.

Journal of Agricultural Science and Technology A 4 (2014) 169-176
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Viability of Extended Goat Semen Stored at Refrigerated Condition

Flocerfida Pagador Aquino[1], Krystel Grace Vergara[2], Lerma Cajuigan Ocampo[1] and Eufrocina dela Peña Atabay[1]

1. Reproductive Biotechnology Unit, Philippine Carabao Center, National Headquarters and Gene Pool, Science City of Muñoz, Nueva Ecija 3119, Philippines

2. College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3119, Philippines

Received: September 12, 2013 / Published: February 20, 2014.

Abstract: Due to the small volume of goat semen ejaculate, just a few doses of goat semen were produced when the sperm concentration is 100 × 10^6/mL. The study was aimed to determine the viability of extended goat semen at refrigerated condition at 5 °C using varying sperm concentrations and evaluated if sperm concentration lower than 100 × 10^6/mL would affect the motility, viability and sperm morphology at refrigerated condition. Using an artificial vagina, ejaculated goat semen was collected from goat semen donor aged 1.5 year. Physical evaluation of the collected semen showed an average volume of 0.54 mL, mean pH of 6.8 and a milky white color with thick consistency indicative of high concentration. Fresh goat semen had an initial average of 76% with an average initial sperm concentration of 128 × 10^6/mL. The semen was divided into four treatments: sperm concentration of 100 × 10^6/mL, 75 × 10^6/mL, 50 × 10^6/mL and 25 × 10^6/mL, and were stored at 5 °C for a period of 10 d. The semen evaluation was performed for each of the four treatments every other day. Results showed that the sperm concentration of spermatozoa affected the duration of storage based on the sperm motility percentage, viability and morphology of spermatozoa. The extended goat semen with sperm concentration of 25 and 50 million sperm/mL is optimal for storage within 6 d that gave satisfactory percentage motile, viable and morphologically normal spermatozoa.

Key words: Extended semen, goat semen ejaculate, motility, sperm concentration.

1. Introduction

Goat is one of the most common and widespread domestic animals. Goat production has importance in agriculture economy worldwide. Breeding programs have been developed for the genetic improvement of goats. One of these programs is the development of artificial insemination (AI) to develop high quality genetic of sires [1]. In addition, the ultimate productivity of the sire depends on the number of sperm production [2]. AI may be regarded as a first generation advanced assisted reproduction technology (ART) and it is the one that has made the greatest contribution to genetic improvement programs, mainly due to well-established methods for identifying males with the highest genetic merit. Three techniques of insemination (vaginal, cervical and intrauterine) are used worldwide in goats [3]. One of the technologies developed in optimizing the viability of animals semen was through refrigeration. The use of refrigerated semen has resulted in higher pregnancy rates when deposited at the cervix compared with frozen semen [4]. Refrigeration of goat semen is particularly important because of its relatively small volume compared to other livestock species such as cattle, buffalo and pigs. The semen volume ranges from 0.5 mL to 1.5 mL with a sperm motility of about 72%-85% and a sperm concentration that ranges from 2.5 × 10^9 sperm/mL to 5.0 × 10^9 sperm/mL [5]. Unlike bulls, buck insemination contains high number of spermatozoa with a suitable semen extender for preservation [6]. Buck ejaculates are small in volume

Corresponding author: Flocerfida Pagador Aquino, Ph.D., research field: animal reproduction. E-mail: floaquino@yahoo.com.

with high concentrations of spermatozoa [7]. Minimal number of straws was produced when the sperm concentration used was 100×10^6/mL [8]. Moreover, semen preservation becomes a necessity when a donor buck is genetically of high quality. Techniques for dilution and frozen storage of goat semen are well advanced but the subsequent fertility can be unpredictable [9].

Refrigeration has been successfully done on buffalo semen with high fertilization rates. Improved motility of spermatozoa and increased preservation time are achieved in other species with chilled extended semen, but there are no reports of its availability in goats. Presently, goat semen refrigeration has renewed the interest among local scientists because of the influx of a variety of introduced breeds of goats. Under local animal management conditions, it becomes necessary to determine the ability of these bucks to become potential semen donors for AI using extended semen in chilled conditions. However, it is essential that an optimum semen concentration with a prolonged viability at refrigerated condition be established by conducting basic research experimentation. Once this is achieved, the extended semen can be used for AI in a group of goats that variably exhibit estrus for a given period even after treatment with synchronizing hormones.

2. Objectives

Generally, the study was aimed to determine the viability of extended goat semen stored at refrigerated condition.

Specifically, the study determined the following factors:

- Motility percentage of extended goat semen with different sperm concentrations;
- Storage duration of the chilled semen with varying sperm concentrations;
- Number of live and dead sperms cells;
- Number of normal and abnormal sperms cells.

The scope and limitation of the study is that the study focused mainly in determining the optimum sperm concentration of extended semen and the duration by which it can be stored at refrigerated temperature with an acceptable number of potentially viable spermatozoa for use in AI.

3. Materials and Methods

3.1 Semen Collection

One healthy Anglo Nubian aged 1.5 years old was used as the semen donor. Semen collection was done once a week early in the morning. Before the actual collection, the pubic region and the prepuce of the buck were cleaned with wet tissue papers.

An artificial vagina (AV) was filled with warm water close enough within the body temperature of the donor animal to simulate a warm environment once the penile organ is inserted into it.

A teaser doe was used to stimulate for the donor to mount to facilitate semen collection. With the use of an AV, the ejaculate was collected in a sterile conical tube that was inserted at the tapered end of the AV. The first squirt of the ejaculate was discarded and only the first and second ejaculates were collected to avoid contamination.

3.2 Handling and Transport of Semen

During the transport of semen sample the conical tube was tucked inside the clothes of the collector to keep it warm. Direct exposure to sunlight was likewise avoided to maintain the viability of the semen.

3.3 General Evaluation of Semen

The semen sample was evaluated initially taking into consideration the semen volume, color, consistency and pH which were all properly recorded.

3.4 Microscopic Evaluation of Semen

The following parameters together with the general evaluation of semen were regularly being done because the effectiveness of the results lies here. Checking these parameters is a means to discard the

donor male that continuously produces poor quality semen.

3.4.1 Motility of Spermatozoa

Sperm motility is an indicator of the spermatozoa quality. The motility rate of the fresh semen samples in this study was found to range between 60% and 80% with an average of 76%. The values obtained indicated a good semen quality based on the standards of sperm motility [10].

Progressive individual motility is one of the major criteria of semen quality [11], and is an important determinant of the success rate of the fertilization. In this study, progressive individual motility was consistently observed in the fresh semen samples collected.

The motility of the semen was evaluated using the wet mount technique with the aid of an inverted microscope (Nikon Eclipse T×10i). The semen was examined for sperm movement demonstrated by the progressive and wavy motion in at least five microscopic fields. Microscopic evaluation was undertaken at low power (10×) and medium (20×) magnification. The scoring system for the evaluation of the movement of sperm was done using the protocol established in Ref. [10].

The semen ejaculate that passed the motility evaluation was divided into four for the following treatments: 100×10^6, 75×10^6, 50×10^6 and 25×10^6 sperm concentrations.

3.4.2 Sperm Concentration

Sperm concentration is considered as an initial indicator of semen quality for cryopreservation [12]. A positive interaction between sperm concentration and semen collection and motility has been reported [13].

A red blood cell (RBC) pipette was filled with semen sample up to the 0.5 mark followed by the diluting fluid up to the 101 mark. The diluting fluid consisted of 3% saline with eosin stain. The mixture in the RBC pipette was mixed well after which few drops were discarded then semen mixture were dropped in the two counting chambers of the haemocytometer.

The number of spermatozoa in four corner squares and the center of the two counting chambers of the haemocytometer were counted and got the average. This is the concentration of spermatozoa per milliliter of semen multiplied by 1×10^7.

Volume of tris-yolk-glycerol (TYG) extender was computed based on the semen volume, sperm concentration and motility.

3.4.3 Evaluation of Live and Dead Sperms

The number of live and dead spermatozoa was determined using eosin-nigrosin as the staining solution. A mixture of 1% eosin and 5% nigrosin in 3% sodium citrate as the standard solution was used to stain the semen smear. The dead sperms absorbed the stain and appeared pink/purple and those that did not absorb the stain are the live sperms. At least 500 spermatozoa were counted in different microscopic fields.

The number of live and dead sperms was counted and the percentage of live and dead sperm was calculated over the total number of sperms observed multiplied by 100 [10].

Table 1 Scoring system for the motility of sperm cells [10].

Motility (%)	Grade	Characteristics
91-100	Excellent motility	90% or more of the spermatozoa is very rigorous in motion. Swirls caused by the movement of the sperm are extremely rapid and constantly going forward progressively.
76-90	Very good motility	Approximately 75%-90% of the spermatozoa are in vigorous rapid motion. Waves and eddies form rapidly but not so rapid as in excellent motility.
60-75	Good motility	About 60%-75% of the spermatozoa are in motion. Motion is vigorous but waves and eddies formed move slowly across the field of vision.
40-59	Fair motility	From 40%-55% of the sperm is in motion. The movements are largely vigorous or eddies are formed.
Less than 40	Poor motility	Less than 40% of the sperm is in motion. The motion is not progressive and mostly weak.
0	Zero motility	No motility is discernable.

3.4.4 Sperm Morphology

Two smears from each semen treatment were prepared which were used for live-dead sperm ratio and the percentage normal-abnormal spermatozoa. This was done by smear staining technique using eosin-nigrosin stain (1% eosin and 5% nigrosin in 3% sodium citrate solution). The shape of the head, neck or middle piece and the tail are examined. Sperm cells with abnormal morphology were counted. At least 500 spermatozoa were counted in different microscopic fields. The percentage abnormal sperm are calculated over the total number of sperm observed multiply by 100 [10].

3.4.5 Procedure for Computing the Final Volume of Extender to Be Added

Calculating the desired volume of extender was done using the formula as follows [9]:

Assumption:

Volume of semen (mL) = 0.20 mL volume/tube

Initial sperm concentration = 144×10^7

Motility score = 70%

Desired final sperm concentration = 10×10^7

Volume of extender = (volume of semen (mL) × % motility × sperm concentration/mL)/(10×10^7) – volume of semen

= $0.20 \text{ mL} \times 0.70 \times 144 \times 10^7/(10 \times 10^7) - 0.20 \text{ mL}$

= 1.816 mL

= 1.816 mL – 0.40 mL (volume of initial extender) (1:2 semen:extender ratio)

=1.416 mL (total volume of extender)

3.4.6 Semen Cooling

After getting semen sample for motility evaluation and sperm concentration, 1:2 dilution (semen:extender) was done. The extended semen and the extender were placed in a stybox with ice. When the temperature inside the styrobox was stable at 10 °C, addition of the volume of extender to be added for each of the treatment was done. After the final dilution, each of the extended semen was carefully mixed well to ensure the homogeneity of the mixture. Once the temperature of the extended semen dropped to about 5 °C, the different parameters for checking sperm viability (motility, live-dead and normal-abnormal sperm) was performed immediately which is recorded as Day 0 observation. The procedure was repeated every other day until the 10th day hereto referred to in series as Day 2, 4, 6, 8 and 10.

3.5 Statistical Analysis

The data collected were subjected to complete randomized design in split plot method with Duncan's multiple range test to compare means at 5% level of significance.

4. Results and Discussion

Table 1 presented the scoring system for the motility of sperm cells [10]. The percentage motility of the sperm cells collected and processed was scored based on the vigorous, swirling movement and the forward progressive movement of the spermatozoa. Those semen samples with 90% or more of the characteristic movements of the spermatozoa were rated 91%-100% motility and graded with excellent motility whereas those semen samples with 76%-90% with the said semen characteristic movements were rated 75%-90% and graded with very good motility. Good motility semen samples were semen with 60%-75% spermatozoa which were in motion although the movement was slowly across the field of vision. Those semen samples with less than 40% of the spermatozoa in motion were rated 40%, with poor motility and characterized with weak motion and therefore unfit for processing. There were also semen samples which were discarded after initial evaluation as there was no sperm movement detected.

Table 2 showed the percentage motility of sperm cells of extended goat semen with varying sperm concentrations. The motility rates did not differ among the four treatments after the initial dilution and immediate cooling at 5 °C (Day 0). After 2 d of refrigeration there was a decline in the percentage

Table 2 Percentage motility of sperm cells of extended goat semen in different sperm concentrations.

Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
100×10^6	73.00^{a}	47.00^{ab}	34.00^{bc}	22.00^{bc}	10.00^{cd}	2.60^{d}
75×10^6	74.00^{a}	54.00^{ab}	37.00^{bc}	28.00^{bc}	19.00^{cd}	5.40^{d}
50×10^6	70.60^{a}	64.00^{a}	57.00^{a}	48.00^{a}	19.00^{b}	12.00^{b}
25×10^6	67.20^{a}	60.00^{a}	49.00^{ab}	41.00^{ab}	31.00^{bc}	22.60^{c}

Means having the same letter script within a row (a, b, c, d) are not significantly different at $P < 0.05$ probability level.

motility of the sperm for all the treatments but was noticeably lowest at 47% for the 100×10^6 sperm concentration/mL followed by 54% for 75×10^6 sperm concentration/mL. Treatments with 50×10^6 sperm concentration/mL and 25×10^6 sperm concentration/mL displayed a slightly higher mean motility of 64% and 60%, respectively, compared to the previous two aforementioned treatments after 48 h of refrigeration.

By the fourth day (Day 4) of refrigeration the extended goat semen showed a sharp reduction in the number of live sperm leaving behind 34% and 37% motility rates for the treatments with a 100×10^6 sperm concentration/mL and 75×10^6 sperm concentration/mL, respectively. The sharp drop in the number of motile sperm can be attributed to the small volume of extender that was added based on the sperm concentration which was set and therefore nutrient supply was inadequate for the given population to maintain.

In addition, a thicker population of sperm in the solution with a lesser quantity of extender causes an overcrowded mixture of dead and motile sperm, the situation of which is quite different when a lower sperm concentration is preferred. The volume of the semen extender to be added is greater to have a more diluted population or less concentrated sperm in the mixture. In effect, the nutrient supply is greater for a specific number of sperm in the extended semen.

As for the extended semen with 50×10^6 sperm concentration/mL and 25×10^6 sperm concentration/mL, the decrease in motility scores was minimal by the fourth day of chilled storage to about 57% and 49%, respectively, which is exactly the same as the general statement of Nuti [14] that when semen is extended and stored above 0 °C, sperm cell survival is for a few days duration.

Prolonging the storage beyond the fourth day at chilled temperature for the experimental treatment with the highest (100×10^6 sperm/mL) and the next highest (75×10^6 sperm/mL) sperm population resulted to a more pronounced reduction in the motility with 22% and 28%, respectively.

Sperm concentrations of 50×10^6 and 25×10^6, the percentage live sperm were 48% and 41%, respectively, in the sixth day (Day 6) of refrigeration. By the eighth day of refrigerated storage condition, all the four treatments displayed decreased sperm motility percentages.

Overall, these findings indicated that extended goat semen can be stored within 4 d at a concentration of 25-50 $\times 10^6$ sperm/mL in the refrigerator and will give acceptable number of motile spermatozoa. When semen is extended and stored above 0 °C, sperm cell survival is of a few days duration and sperm fertilizing ability cannot be preserved for more than a few days.

Table 3 presented the proportion of live sperms for all the treatments. The viability rates did not vary ($P < 0.05$) between the four treatments after the initial dilution and immediate cooling at 5 °C (Day 0). After 2 d of refrigeration there was a decline in the percentage of live sperm cells for all the treatments but was noticeably comparable at 58.96% for 100×10^6 sperm concentration/mL and 58.40% for 75×10^6 sperm concentration/mL.

Treatments with 50×10^6 sperm concentration/mL and 25×10^6 sperm concentration/mL displayed a slightly higher percentage of live sperm cells of 64.96%

Table 3 Percentage live sperm cells of extended goat semen in different sperm concentrations.

Trt	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
$100x10^6$	73.32 [a]	58.96 [ab]	36.52 [bc]	28.76 [cd]	17.32 [cd]	12.36 [d]
$75x10^6$	75.04 [a]	58.40 [ab]	47.96 [bc]	33.08 [cd]	24.36 [d]	16.24 [d]
$50x10^6$	69.60 [a]	64.96 [a]	60.56 [a]	52.48 [a]	29.16 [b]	21.16 [b]
$25x10^6$	68.92 [a]	62.00 [ab]	58.32 [abc]	53.80 [abc]	43.40 [bc]	36.20 [c]

Means having the same letter script within a row (a, b, c, d)) are not significantly different at $P < 0.05$ probability level.

and 62% correspondingly. By the fourth day (Day 4) of refrigeration the extended goat semen showed a sharp reduction in the number of live sperm with 36.52% and 47.96% livability rates for the treatments 100×10^6 sperm concentration/mL and 75×10^6 sperm concentration/mL, respectively.

The results of this study might be attributed to the effect of refrigeration temperature of 5 °C to the spermatozoa that lose its viability due to the rapid changes of the temperature that the spermatozoa undergo in what we called cold shock. Another factor could be the components of the extender specifically the egg yolk wherein it was observed that the refrigerated extended semen with high sperm concentrations coagulates upon longer duration of storage period. The dilution of goat semen in extenders containing egg yolk can be deleterious to sperm cells. This occurs because the goat semen has characteristics that differentiate it from other species, being the most important presence of phospholipase A secreted by the bulbourethral glands. This phopholipase is also called Eyce (egg yolk coagulating enzyme) or BUSgp60 (bulb urethral gland secretion; Ref. [3]) and is responsible for the reduced viability of sperm cells that have been cooled in extenders containing egg yolk [15]. As for the extended semen with 50×10^6 sperm concentration/mL and 25×10^6 sperm concentration/mL, the decrease was minimal with a live sperm population to about 60.56% and 58.32%, respectively.

Extending the storage for more than four days at chilled temperature, experimental treatments with the highest sperm concentration of 100×10^6 sperm/mL followed by 75×10^6 sperm population/mL resulted to a more evident reduction in the percentage number of live sperm cells with 28.76% and 33.08%, respectively.

As for the treatments with a sperm population of 50×10^6 and 25×10^6, the percentage of live sperm was 52.48% and 53.8%, correspondingly on the sixth day (Day 6) of refrigeration. By the 8th day of chilled extended semen, there was reduction of live sperm with 17.32% and 24.36% for the treatments 100×10^6 sperm concentration/mL and 75×10^6 sperm concentration/mL respectively, 29.16% and 43.40% for the sperm concentration of 50×10^6 sperm/mL and 25×10^6 sperm/mL respectively. The tenth day of refrigeration resulted to rapid decline for the percentage of viable sperm in three treatments except for 25×10^6 sperm/mL with 36.20%.

Taken as a whole, these findings indicated that extended goat semen can be stored within 2-4 d with a concentration range of 25-50 $\times 10^6$ sperm/mL in the refrigerator that gave acceptable number of live spermatozoa.

There were no significant differences ($P < 0.05$) on the proportion of normal sperm cells from all the treatments in the study. However, as the length of storage was continued and analyzed every two days, there was a reduction in the percentage of sperm that were morphologically normal (Table 4). The percentage of normal sperm in the chilled semen was inversely proportional with the percentage of abnormal sperm that were observed as the refrigeration period was extended.

Sperm morphology has been proposed as criteria for the selection of fertile ejaculates [3]. Semen

Table 4 Percentage normal sperm cells of extended goat semen in different sperm concentrations.

TRT	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
100×10^6	73.80^{a}	67.08^{ab}	64.12^{b}	61.64^{bc}	55.48^{cd}	50.84^{d}
75×10^6	75.52^{a}	69.48^{ab}	64.16^{b}	57.00^{c}	53.56^{cd}	49.84^{d}
50×10^6	77.80^{a}	72.24^{ab}	69.44^{b}	61.44^{c}	56.84^{c}	45.84^{d}
25×10^6	72.80^{a}	69.72^{a}	65.72^{ab}	61.20^{bc}	57.96^{c}	47.84^{d}

Means having the same letter script within a row (a, b, c, d) are not significantly different at $P < 0.05$ probability level.

morphology has a limited positive predictive value for field fertility. It, however, is important to screen-out clearly poor-quality ejaculates [16]. Moreover, semen quality decreases over time so that after about 5 d of storage at 5 °C, cooled semen features were equivalent to those of frozen semen [17].

The effect of refrigeration in the normal morphology of the spermatozoa might be based on the adaptive mechanism of individual spermatozoa in chilled condition; weak adoption of the spermatozoa may lead to increasing number of semen abnormalities.

5. Conclusions

Based on the findings and observations on this study, It could be concluded that (1) extended goat semen can be stored in chilled condition for up to two days with concentration either 50×10^6 sperm/mL or 25×10^6 sperm/mL; (2) it was observed that 25×10^6 sperm/mL and 50×10^6 sperm/mL concentrations appear to be the optimal concentrations since they obtained the highest percentage motility, livability and normal sperm morphology for up to four days; (3) prolonged storage of the semen samples resulted to the deterioration of the sperm morphology, rapid decline in motility and decrease in the viability of the spermatozoa in chilled condition which resulted in increasing percentage of dead and abnormal spermatozoa.

The ability of chilled goat semen to fertilize an egg by sperm penetration assay (IVF) is recommended in order to determine its fertilizing potential.

AI in synchronized goats using extended semen within 2-4 d could be conducted.

For better evaluation, the use of computer-assisted semen analysis system (CASA) may be very useful for future investigations.

Use other breeds of bucks for extended semen.

References

[1] J.P. Dubeuf, J. Boyazoglu, An international panorama of goat selection and breeds, Livestock Science 120 (2009) 225-231.

[2] R.L. Ax, M.R. Dally, B.A. Diddion, R.W. Lenz, C.C. Love, D.D. Varner, et al., Artificial insemination, in: B. Hafez, E.S.E. Hafez (Eds.), Reproduction in Farm Animals, Lippincot Williams and Wilkins, A Walter Kluwer Co. London, 1993, pp. 376-389.

[3] B. Leboeuf, B. Restall, S. Salamon, Production and storage of goat semen for artificial insemination, Animal Reproduction Science 62 (2000) 113-141.

[4] S. Salamon, W.M.C. Maxwell, Storage of ram semen, Anim. Reprod. Sci. 62 (2000) 77-111.

[5] D.L. Garner, E.S.E. Hafez, Spermatozoa and seminal plasma, in: Reproduction in Farm Animals, B. Hafez, E.S.E. Hafez (Eds.), 6th ed., Philadelphia, USA, 2000, p. 107.

[6] G. Evans, W.M.C. Maxwell, Salmon's Artificial Insemination of Sheep and Goats, Butterworth, London, 1990, pp. 107-141.

[7] T. Mann, R. Jones, R. Sherins, Oxygen damage, lipid peroxidation and motility of spermatozoa, in: A. Steinberg, E. Steinberg (Eds.), Testicular Development, Structure and Function, Raven Press, New York, USA, 1980, pp. 497-501.

[8] M.A.G. Beltran, Optimized extenders for cryopreservation of buck semen for artificial insemination, Philipp. J. Vet. Anim. Sci. 3 (2013) 1-10.

[9] J.M. Corteel, Production, storage and insemination of goat semen, in: Proceeding of Management of Reproduction in Sheep and Goats Symposium, University of Wisconsin, Madison, 1977, pp. 41-57.

[10] F.V. Mamuad, E.V. Venturina, H. Saito, Collection, processing and handling buffalo semen, Water Buffaloes and Beef Cattle Improvement Project (WBBCIP), A joint

JICA assisted project of the Philippine Carabao Center and the Bureau of Animal Industry, Philippines, 2004.

[11] J.F. Lasley, R. Bogart, Some factors influencing the resistance of bull sperm to unfavorable environmental conditions, J. Anim. Sci. 1 (1942) 79.

[12] V.B. Shelke, A.J. Dhami, Comparative evaluation of physico-morphological attributes and freezability of semen of Gir cattle (*Bos indicus*) and Jafarabadi buffalo (*Bubalus bubalis*) bulls, Indian J. Anim. Sci. 71 (2011) 319-324.

[13] M. Mathevon, M.M. Buhr, J.C.M. Dekkers, Environmental, management and genetic factors affecting semen production in Holstein bulls, J. Dairy Sci. 81 (1998) 3321-3330.

[14] L.C. Nuti, Goat semen collection and processing, http://www.anscicornell.edu/goats/Resources/GoatArticles/GoatGeneticsRepro/GoatSemenNuti.pdf.1-39.

[15] J.M. Corteel, Collection, processing and artificial insemination of goat semen, in: C. Gall (Ed.), Goat Production, Academic Press, London, 1981, pp. 171-191.

[16] G.C. Althouse, Evaluating porcine semen for artificial insemination. Part II: Assessment of cell membranes and viability, Comp. Cont. Ed. Pract. Vet. 19 (1997b) 400-404.

[17] G.C. England, P. Ponzio, Comparison of the quality of frozen-thawed and cooled-rewarmed dog semen, Theriogenology 46 (1996) 165-171.

Journal of Agricultural Science and Technology A 4 (2014) 177-187
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Advantages and Disadvantages of Transgenic Animal Technology with Genetic Engineering

Mine Dosay-Akbulut

Department of Veterinary Medicine and Genetics, Veterinary Faculty, Afyon Kocatepe Univesity, Afyon 03200, Turkey

Received: November 12, 2013 / Published: February 20, 2014.

Abstract: Transgenic animal technology has been one of the fastest growing biotechnology areas. The exogenous genes have been introduced into the animal genome by genetic engineering, so that these genes can be inherited and expressed by offspring to produce desired traits or evaluate function in elite livestock breeds. There are several methodologies for the production of transgenic animals, i.e., (1) microinjection of genes into pronuclei of fertilized ova; (2) DNA transfer by retroviruses; (3) injection of embryonic germ (EG)/embryonic stem (ES) cells previously treated with foreign DNA; (4) DNA transfer into cells and embryos with using liposomes; (5) exogenous DNA transfer while *in vitro* fertilization by using sperm; (6) electroporation of DNA into sperm, embryos or ova; (7) biolistics; (8) nuclear transfer (NT) with somatic cells, EG or ES cells; (9) germ line stem cell-mediated; (10) gene targeting; (11) gene silencing technology with RNA interference; (12) induced pluripotent stem cell; (13) zinc-finger nuclease gene targeting technology. Gene farming is one of the newest and most promising areas in modern biotechnology. Cattle, goats, sheep, pigs and rabbits are the main farm livestock species and fish is also used in transgenic technology. The question of "why make transgenic animals?" is very important. Some of the answers to this question are: (1) to obtain new knowledge; (2) to solve the genetic code; (3) to create genetic disease models; (4) to study the genetic control of physiological systems; (5) to improve animal production traits; (6) to produce new animal products. Transgenic technology is one of the main and important tools in the finding solutions to problems of growing population with their applications to different organisms, and takes more attention and interest every day. Transgenic technology creates opportunities and areas to play with organisms to fulfill the demands of people. Because of this, this paper based on mainly transgenic applications to take people's attention and exhibit its importance.

Key words: Biotechnology, bioreactors, livestock, transgenic, animal, genetic engineering.

1. Introduction

Genetic engineering is the process of modifying an organism's genetic composition by adding foreign genes to produce desired traits or evaluate function. By introducing of "foreign" deoxyribonucleic acid (DNA) into preimplantation embryos, transgenic animals are produced. The foreign DNA is merged with the genetic material and may be expressed in tissues of the obtained result. This technique is of great importance to many aspects of biomedical science including gene regulation, cancer research, the immune system, developmental biology, biomedicine, manufacturing and agriculture.

The production of transgenic animals is one of a number of new and developing technologies that will have a profound impact on the genetic improvement of livestock. The first transgenic animals contained foreign DNA that was passed on to offspring [1]. Transgenic technology represents a revolutionary way to produce elite livestock breeds, allowing introduction of alien gene into livestock genome [2]. Transgenic animal technology is one of the fastest growing biotechnology areas. It is used to integrate exogenous genes into the animal genome by genetic engineering technology so that these genes can be inherited and expressed by offspring. The transgenic efficiency and precise control of gene expression are the key limiting

Corresponding author: Mine Dosay-Akbulut, associate professor, research field: molecular and systematic genetics. E-mail: minedosay@aku.edu.tr.

factors in the production of transgenic animals. A variety of transgenic technologies are available. Each has its own advantages and disadvantages [3].

Transgenic animals have huge applications in the improvement of animal production quality, the increase of production capacity, the studies related to human disease models and the production of biomedical materials [3].

A transgenic or genetically modified organism is one that genetically altered via recombinant DNA technology, which includes either the joining of DNA from different genomes or the insertion of foreign DNA into a genome. Actually, the entire biotechnology industry is based on the capacity of added new genes to cells, plants and animals [4].

The question of "why make transgenic animals?" is very important. Some of the answers to this question are: (1) to obtain new knowledge, (2) to solve the genetic code, (3) to create genetic disease models, (4) to study the genetic control of physiological systems, (5) to improve animal production traits and (6) to produce new animal products. The production of transgenic organisms has been a major technical advance in the study of biology. It is an important method in playing with the genetic make-up of an animal providing mutation and species crossing technology. Transgenic animals have been an important tool in providing new insights into the study of the mechanisms of gene regulation and developmental biology [5]. Other areas that transgenic technology has also provided significant advantages and exciting future possibilities are: (1) the action of genes implicated in the development of cancer mechanisms; (2) the mechanisms of regulation and cell interaction in the immune system; (3) as models for human genetic diseases; (4) the mechanisms and growth control; (5) the main mechanisms of biology and genetics [6]; (6) in medical research, transgenic animals are used to determine the functions of specific factors within the homeostatic systems via over- or under-expression of a modified gene (the inserted transgene); (7) in toxicology, as sensitive test animals (detection of toxicants); (8) in mammalian developmental genetics; (9) in molecular biology, determination of the regulation of gene expression which help us in the evaluation of a specific genetic change at the level of the whole animal; (10) in the pharmaceutical industry, aimed production of pharmaceutical proteins, drug production and productivity testing; (11) in biotechnology, producing of specific proteins to increase milk yield producing of genetically engineered hormones and meat production; (12) efficient modification of animal physiology and/or anatomy; (13) in cloning, to reproduce specific blood lines; and (14) developing of special animals to use in xenografting [7].

2. Materials and Methods

Mainly three methods were used for the creation of transgenic animals as follows:

(1) DNA microinjection. It is based on the direct microinjection of a targeted gene construct (a single gene or a combination of genes) from another member of the same species or from a different species, into the pronucleus of a fertilized ovum. The main advantage of this method is their application to a wide variety of species.

(2) Embryonic stem cell-mediated gene transfer method. It involves primarily introducing of the desired DNA sequence by homologous recombination into an *in vitro* culture of embryonic stem (ES) cells. The offspring animals are called chimeric animal. ES cell-mediated gene transfer method can be adapted to gene inactivation, with so-called knock-out method. This technique has a particular importance for the study of the genetic control of developmental processes. It is possible to detect precisely mutations in the gene via homologous recombination.

(3) Retrovirus-mediated gene transfer method. It is based on the gene transfer with the help of the carrier or vector, generally a virus or a plasmid. Generally, retroviruses are used as vectors to transfer genetic

material into the cell, with its ability to infect host cells aiming higher expression probability. The offsprings are chimeric as well [7].

Animal transgenic technologies include gene targeting to improve accuracy, the germ line stem cell-mediated method to improve efficiency, zinc-finger nuclease gene targeting technology, RNA interference-mediated gene silencing technology and induced pluripotent stem cell technology [1]. Gene farming is one of the most promising areas in modern biotechnology. The use of live bioreactors for the expression of human genes in the lactating mammary gland of transgenic animals seems to be an important method for the production of valuable recombinant therapeutic proteins. Also, it is possible to evaluate known growth factors and their receptors and modulators using transgenic technology. Transgenic farm animal production has found broad acceptance in biomedical applications. Cattle, goats, sheep, fish, pigs and rabbits are the main farm livestock species that are used in transgenic technology, with aiming expression of tens to hundreds of grams of genetically-engineered proteins or xenogeneic biopreparations in their milk [2]. With the research and development of genetically engineered animals (GEAs) in breeding of new variety, xenotransplantation, bioreactor and disease model, biosafety issues of GEAs have attracted widespread attentions worldwide. To regulate research and application of GEAs or similar products, governments and agencies have established related laws and regulations [8].

Lentivector technologies now have widespread use in basic biology and translational studies including transgene overexpression, persistent gene silencing, *in vivo* imaging, immunization, generating transgenic animals, stem cell modification, induction of pluripotent cells and lineage tracking or site-directed gene editing. Moreover, in the present high-throughput "-omics" era, the availability of lentiviral vectors, which are engineered to express or silence genome-wide genes, accelerates the rapid expansion of this vector technology [9].

Lentiviral vectors introduce several attractive features as gene-delivery vehicles, including: (1) regular gene delivery via stable vector integration into host genome; (2) the characteristics of infecting both dividing and non-dividing cells; (3) broad tissue expansion, including important gene- and cell-therapy-target cell types; (4) unexpressed viral proteins after vector transduction; (5) delivering ability of complex genetic elements, such as polycistronic or intron-containing sequences; (6) providing safer integration into right places; (7) a much easier system for vector manipulation and production [10]. There are several methodologies for the production of transgenic animals, such as (1) microinjection of genes into pronuclei of fertilized ova; (2) DNA transfer by retroviruses; (3) injection of embryonic germ (EG)/ES cells previously treated with foreign DNA; (4) DNA transfer into cells and embryos with using liposomes; (5) exogenous DNA transfer while *in vitro* fertilization by using sperm; (6) electroporation of DNA into sperm, embryos or ova; (7) biolistics; (8) nuclear transfer (NT) with somatic cells, EG or ES cells; (9) the germ line stem cell-mediated method; (10) gene targeting; (11) gene silencing technology with RNA interference; (12) induced pluripotent stem cell; (13) zinc-finger nuclease gene targeting [6].

"Translational Medicine" in a human health research became a top priority. Suitable animal models in the evaluation of utility and safety of new drugs or therapeutic structure are important for the success of translational research. In this sense, rodent models are generally used for suitable to genetic and environmental standardization, providing answers to specific scientific problems with a broad spectrum and their acceptance by the regulatory authorities. But creating and using transgenic pigs get more attention recently as an alternate to the rodents, because of their similarities with human in anatomy, physiology,

metabolism and pathology. Also pigs as large animal models can be preferred in transgenic studies, because of their reproductive talent, a short generation interval period, multiple offspring birth and all season breeding possibilities. Moreover, the sequence data indicate that the pig is closer to human than mouse. This result was supported by the comparative analysis of protein coding sequences using full-length cDNA alignments from human, mouse and pig. At present, transgenic pigs largely created as an animal model of some human diseases, such as neurodegenerative diseases, cardiovascular diseases, cystic fibrosis and diabetes mellitus [11].

An important application of transgenics is the production of therapeutic proteins for human clinical use called “bio-reactors”. By using genetic engineering it has become possible to produce any protein from any animal, plant or bacterial species in the milk of mammals [5]. The process of transgenic goat production with human protein can be seen in Fig. 1. With this method, human protein can be obtained from transgenic goat milk.

The transgenic livestock production has been an important tool, providing a new approach into the gene action mechanisms related to the control of growth. It is possible to do some changing in known growth factors, growth factor receptors and growth modulators via transgenic technology.

Another important approach to the agricultural transgenics is increasing possibility in disease resistance via introducing specific genes into livestock.

Identification of single genes within the major histocompatibility complex (MHC), which affects the immune response, is a tool for the determination of the genetic basis of disease resistance and susceptibility. The application of transgenic methodology into the immune system should provide an opportunity to obtain genetically engineer livestock with superior disease resistance.

Embryo biotechnology has evolved through three major changes which are traditional embryo transfer (*in vivo* embryo production by donor superovulation), *in vitro* embryo production by ovum pick up with *in vitro* fertilization and notably current cloning technique by somatic cell nuclear transfer and transgenic animal production [10].

The cloning technique provides different alternative possibilities to the stem cell studies. The stem cells, obtained from cloned embryo, will supply new advantages to the treatment of different uncured diseases. The stem cells can be taken from the bone marrow and infants’ bellybands, but it is limited to specific tissues, creating problems in transfer to other tissues. However the stem cells, taken from the cloned embryo, can transform into any tissues easily without rejection of the newly-produced organs or tissues by the body [12].

The first transgenic livestock was produced by microinjection of foreign DNA into zygotic pronuclei in 1985. Typical applications include carcass composition, lactational performance and wool production with also enhanced disease resistance and reduced environmental impact. Production of transgenic animals has great application in agriculture

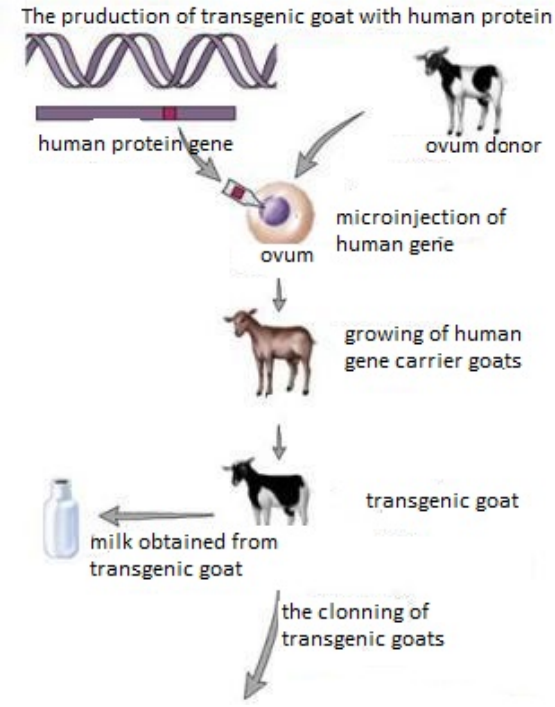


Fig. 1 Transgenic goat producing steps.

and medicine. In beef cattle industry, the transgenic technology can be used in developing animals for faster growth, higher quality beef products or disease resistance.

Transgenic technique showed that by adding a single growth regulating gene into an animal of agricultural value while animal growth rate and feed efficiency could be increased and in contrast to this, fat deposition could be reduced. Many other applications such as enhanced milk production with novel properties, enhanced disease and parasite resistance and increased wool production can be prepared by using this technique, creating big changes in meat animal industry [10].

Transgenic farm animal production for biomedical applications has found broad acceptance. Genetic modifications including lentiviral transgenesis, meganucleases, small interfering ribonucleic acids, somatic cell nuclear transfer, zinc finger nucleases and transposons [13]. Somatic cell nuclear transfer and pronuclear microinjection of DNA are two important methods used to make transgenic farm animals [14]. These new strategies also include the use of bacterial products such as proteins, immunotoxins, enzymes and secondary metabolites which specifically target cancer cells and cause tumor reduction through growth inhibition, cell cycle arrest or apoptosis induction [15].

Retroviruses are viruses that insert a DNA copy of RNA into the host cell DNA afterwards infection. The retrovirus serves as a naturally occurring delivery system to transfer DNA into different types of mammalian cells. Preimplantation embryos or oocytes can be subjected to concentrated virus solutions or incubated with virus-producing cells as *in vitro*. After exposure to the viruses, *in vitro* infected embryos are transferred back to receiver females to complete the gestation. Introducing of foreign genes into animals can be managed by also pronuclear injection, which is another method. Transgenic mice, has been produced successfully mostly by microinjection of cloned DNA into the pronucleus of a fertilized ovum [6].

The progress on the transgenic chicken technology has been delayed in contrast to mammalian species. There are two reasons for this delay, first one is that only one-cell-stage oocyte can be obtained from a sacrificed hen and the other reason is that the yolk inhibits the precise observation of oocytes in microscope. Recently, new methods have been developed to obtain successfully transgenic chickens. Mostly retroviral vectors are used because of their success in cooperation of transgenes into host cell chromosomes. These viral vectors are injected into the embryos at blastodermal stage directly, and then primordial germ cells (PGCs) are infected *in vitro* and implanted into recipient embryos [16].

The first reports of transgenic livestock presented the introduction of growth-promoting genes into pigs and sheep. To obtain further increase in swine, transgenic pigs have been generated with overexpress of bovine a-lactalbumin in their milk [17]. Levels of a-lactalbumin in milk can effect milk production and litter weight positively. Transgenic pigs have been generated also to deal with the environmental issue of manure-based phosphorous pollution. Because swine do not have an ability for breaking down dietary phosphorous, transgenic pigs by producing a bacterial phytase gene in their saliva lead to 75% reduction in fecal phosphorous. Also transgenic modifications in the fat content of goat milk and casein levels of cow milk have been reported. The consumer has an advantage from the lowered unhealthy saturated fats and the higher degree of casein in milk effecting positively cheese yields. Recently, a transgene in dairy cow has been reported to act on disease resistance. In near future, we will see the animals generated with endogenous gene modifications, using nuclear transfer techniques. For example, knocking out the expression of unwanted protein or correcting a genetic disease is now possible [18].

Two new transgenic methods have been getting attention in transgenic livestock technology which are:

(1) the isolation and obtaining of embryonic and somatic cells directly from *in vitro* embryos, fetuses and adults and (2) the use of these embryonic and somatic cells as nuclei donors in NT or "cloning" strategies. These embryos are called chimeras. Introducing of exogenous DNA into animals with aiming of producing transgenics can be made by different vehicle successfully. One of them is sperm-mediated gene transfer. Successful sperm-mediated gene transfer has been determined in the mouse. Liposome/DNA delivery methods also can be used for introducing cloned DNA into cells and embryos. Liposomes are small vesicles including membrane like lipid layers that have an ability to protect foreign DNA from digestion of proteases and DNases. The transfer of cloned DNA into cells and embryos has been managed with using electroporation method. In delivering DNA or other molecules into intact tissues and cells, biolistics or particle bombardment can be used via microprojectiles. It is possible to increase the number of offspring from a single female into the tens of thousands by using NT (cloning) techniques. The famous cloned sheep "Dolly" was born with NT technology, also another methodology has been developed for transgenic animals [6].

The new method, used in the production of genetically identical individuals from embryonic and somatic cells via NT provides an opportunity to the rapid development of genetically identical animals with a targeted gene insertion. The two basic strategies are used in producing of transgenic animals. These are the so-called "gain of function" or "loss of function" [6].

The gain of function paradigm depends on that by adding a cloned fragment of DNA to an animal's genome, which you can manage several new targets. One goal is to obtain new gene expression that previously not existed in that cell or tissue type, for example, enabling the new expression of human growth hormone (hGH) in mouse liver. Another possible strategy is the use of DNA constructs which encode a messenger RNA in the antisense or reverse orientation on the basis of the promoter. This antisense RNA binds to the sense mRNA strand, transcribed from the animal's own DNA, constitute a heteroduplex which inhibits cytoplasmic translation of the protein. Lastly, this gain of function strategy has been used to disrupt an endogenous gene by insertion into the host's genome [6]. Integration of foreign DNA will most probably affect many genes and stimulates new mutations with creating duplications, deletions or more complex chromosomal rearrangements and translocations [19]. This insertional mutagenesis with integration into a functional region of the host genome has been obtained by chance and therefore cannot be planned. But about 5% of the transgenic animals will come up with similar mutations.

The "loss of function" paradigm has many similarities with the "gain of function" strategy especially on the regard of over expression, insertional mutations and antisense situations. This strategy is based on the ability of embryonic cells to undergo homologous recombination. The transgenic animals produced with the ability of the cell to form stable recombinants between the exogenous DNA and the endogenous chromosomal DNA to the host's genome. This is called homologous recombination, or in other words "gene targeting". Gene targeting allows *in vitro* transfer of genetic alterations into specific sites in the embryonic or cell genome. If the host's cells are pluripotent or totipotent embryonic cells (i.e., ES, EG cells and PGCs) or re-programmable somatic cells, then these homologous recombination experiences can be transferred to the germ line of the offspring [6].

3. Some Important Transgenic Applications' Samples

Transgenic mammalian species constituted with somatic cell cloning will have using possibility in biomedicine, the biopharmaceutical industry, human

nutrition/dietetics and agriculture. In mammals, somatic cell cloning technology improves the multiplication of productively-valuable genetically engineered individuals, and as a result allows for standardization of transgenic farm animal-derived products which is in the market demands, will have growing significance [20]. In bioreactors, mammalian cells as production host are the focus in last years. The production of iPSCs (induced pluripotent stem cells) from livestock or wild species is getting more attention because it could improve efficiency and reduce costs in various fields, such as transgenic animal generation, preservation of biological diversity and drug development, and propose an alternative to xenotransplantation for *in vivo* generation of organs [21].

Transgenic rodents are also important in the studies. For example, Chinese hamster ovary (CHO) cells are taking interest in the manufacture of recombinant therapeutic proteins. CHO cells are the manufacturing host system of choice for more than 70% of protein pharmaceuticals on the market [15]. Transgenic mice have revolutionized biology, medicine and biotechnology in the 21st century [1]. In mammals aging studies, the mouse is a perfect model due to its relatively short life time, and the genetic manipulations in this species are well established. Most interestingly, the mouse can be used in the producing of the stem cells. It will be necessary to establish iPSCs (pluripotent stem cells) from their tissues to treat damaged tissues or repair organs in elderly patients. It has been succeeded in establishing iPSC clones using bone marrow (BM) from 21-month-old EGFP-C57BL/6 mice [17]. Although genetically modified mice have been widely used to model human diseases, some of these mouse models do not provide the same symptoms or pathology. Pigs are more similar to humans than mice in anatomy, physiology and genome. Because of this, pigs are considered more suitable animal models to mimic some human diseases [22].

The use of bacteria in the regression of certain forms of cancer has been recognized for more than a century. This includes the use of attenuated bacterial strains and expressing foreign genes that encode the capacity of converting non-toxic prodrugs to cytotoxic drugs [14].

Spider silk is an important biomaterial with many applications in biotechnology and biomedicine, and this material has several desired characteristics such as outstanding strength, toughness, elasticity, biodegradability and biocompatibility. There has been a higher effort to produce recombinant spider silk protein in large amounts [23].

The zebrafish has been used for a long time as a model organism in developmental biology studies. In fact several common and important developmental mechanisms have been identified in zebrafish which are similar in mammals. The zebrafish has short generation time and their embryos are transparent and therefore provide unique imaging opportunities. They can be used also as a model organism for several pathophysiological conditions which are related to human diseases. For instance, zebrafish is used as an inflammation and regeneration model because of its ability to partially recovery for organ loss (e.g., heart and fins). It is also used in tumor biology, for drug screening, systems biology, congenital and hereditary disease and in infection [24]. It is also important to use zebrafish for AD research, because it represents a more global cross-species modelling (from fish to rodents) to detect uncovering evolutionary conserved mechanisms of neurodegeneration. Indeed, zebrafish represents a lot of order behaviors including memory, conditioned responses and social behaviors like schooling. All these structures support the zebrafish as an ideal model for studying human diseases, including CNS disorders [25].

GloFish is a type of transgenic zebrafish (*Danio rerio*) that have been obtained via insertion of a green fluorescent protein (GFP) gene [4].

Many marine fish species synthesize antifreeze

proteins (AFP) to protect them from freezing within the icy waters. The winter flounder AFP genes were transfered successfully into Atlantic salmon. The results determined stable genomic integration and low levels of expression of winter flounder AFP genes in a small number of transgenic salmon developed by microinjection. Low levels of AFP precursors were obtained from the blood of all transgenic offspring (F1) [26].

Transgenic technique provides a lot of alternative methods for fish breeding. Up to now, the growth hormone gene transfer to carps, salmon and tilapia as well as fluorescence protein gene transfer to zebra fish and white cloud mountain minnow has been successfully carried out. The higher amount of GH hormone production via GH gene transfered transgenic fish will effect and promote the aquaculture production and economic efficiency [27].

Transgenic salmon with their incresed growth rate could be on the market when their movement and escape will be controlled in oceans [28].

By using genetic engineering it has become possible to produce any protein from any animal, plant or bacterial species in the milk of mammals [5]. For example, it is possible to produce milk proteins and other proteins of pharmaceutical value in the milk of rabbits, mice, pigs, goats and sheep [6].

The idea of using genetically modified animal organs, tranplantation into humans was developed. The pig has become the obvious source of these genetically modified organs because pig's organs are anatomically and physiologically more suitable with humans [6].

Transgenic animals may also be very useful for the testing of new drugs or products. Transgenic rodents that are more sensitive to environmental toxins are able to test new drugs, products or materials for safety much more quickly than their wild-type counterparts [6].

The development in recombinant DNA technology has provided the opportunity either to produce entirely novel proteins in milk or to change the composition of milk. These changes cause an increase in the value of milk and the potential uses of milk. The improvement of livestock growth or survivability via the modification of milk composition demands production of transgenic animals that: (1) produce milk of higher nutrient content; (2) produce a greater quantity of milk or (3) produce milk that contains a beneficial "nutriceutical" protein. The major nutrients in milk are protein, fat and lactose and the increase of these components can affect the growth and health of the offspring [6].

An important transgenic sheep called Tracy obtained via micro-injection produced milk with over 50% of the protein, including human alpha-1-anti-trypsin. Sheep-derived protein has been used in clinical test for congenital emphysema in UK. Another important animal, Polly, a cloned sheep obtained via nuclear transfer, born with the human gene encoding factor IX, produced a protein involved in preventing haemophilia [12].

Transgenic mice, sheep and pigs have been used to search postnatal growth of mammals. For example, the *myostatin* gene of mouse is an extraordinally amazing potential locus for "knock-out" by ES cells in meat producing species. The loss of the myostatin protein causes an increase in lean muscle mass. Mice lacking of this gene have increased shoulders and hips. This enlarged skeletal muscle mass can be seen in whole carcass and appears grossly normal. Another approach in manipulating carcass composition is the modification of the fat or cholesterol composition of the carcass. It is possible to decrease meats, eggs and cheeses' fat and cholesterol content by changing the metabolism or uptake of cholesterol and/or fatty acids.

There is also the possibility in obtaining healthy fats such as the omega-3 fatty acids from fish into our livestock.

By manipulating the enzyme profiles in the gut or increasing the uptake of nutrients in the digestive tract, it can affect the feed efficiency affirmatively.

Another important approach to the agricultural transgenics is that increasing possibility in disease resistance via introducing specific genes into livestock.

The application of transgenic methodology into the immune system should provide an opportunity to obtain genetically engineer livestock with superior disease resistance. One example of this application is the production of trangenic pigs resistant to influenza [6].

Introduction of polymorphic *ESR* gene could increase the litter size in different breeds of pigs. Another example, but in merino sheep was reported by Piper et al., 1985, the authors found that the *FECB* gene, one single major autosomal gene for fertility, provided the increasing of ovulation rate [29].

Another important subject of the transgenic manipulation in livestock is the control of the quality, yield, color and simplest harvest of hair, wool and fiber for fabric and yarn production. The manipulation of the quality, fineness, length and crimp of the wool and hair fiber from sheep and goats has been verified with transgenic methods.

Also, it is possible to use transgenic methods for increasing elasticity of the fiber and the fiber strength. The scientist is planning to use transgenic manipulation on the surface of the fibers, related to the wool for the future. The reduction on the surface interaction could decrease shrinkage of garments made from these fibers. Also, there is big effort to use this technology in collecting shed of wool, from sheep, into specific times for relieving in especially hand shearing of fiber producing animals. Similar strategies could be adapted to mohair goats, alpacas, camels and other fiber-producing animals [6].

Avian species have emerged as a model system to study the neurobiological problems on the basis of complex behavior. Some important studies in this context are that genetically modified hens that produce therapeutic proteins in their eggs and transgenic songbirds that can be used as a model to study communication disorders and many neurobiological features such as the biological basis of vocal learning. The rich resources of songbird behavior suggests that these species can be used as an ideal animal model for human diseases affecting complex cognitive functions and communication disorders, which cannot be modeled completely from other animals.

Chickens can be used as animal bioreactors with a lot of advantages, i.e., (1) their eggs are extremely rich sources of protein (6 g in 45 mL of total volume), and egg contents are naturally sterile; (2) transgenic chickens need less time to mature (21 d to hatch, 5-6 months to reach sexual maturity) compare to large livestock such as goats or cows. Thus using transgenic chickens affect the cost and duration of the development process with decreasing; (3) chicken eggs can be used extensively in the manufacturing of therapeutic proteins, biopharmaceuticals and in vaccine production [30].

4. Look into Transgenic Technique with Ethical Issue on the Basis of Their Advantages and Disadvantages

Transgenic animals can be used for the improvement of animal production quality, the enhancement of production capacity, the studies of human disease models, and the production of biomedical materials. But there are some problems that need to be resolved related to transgenic animal studies. First of all, the transgenic technique is not perfect with low success and survival rates of transgenic animals. These are the main determinant in the transgenic animal studies. Secondly, the joining efficiency of external genes at the determined site is low and unstable, and the effect to the intrinsic gene creating abnormalities in animals is unclear. Thirdly, this technique is still in immature stage which means it requires further studies. Also, there are safety concerns in the production of transgenic animals as well, for example, inserted external genes may affect

the host with contamination of other genes, and produce a lot of threats to ecological balance and species diversity [3].

The production of transgenic animal may lead to food-safety problems like allergies or toxicities. Because of these, serious considerations related to the safety of transgenic animals and modification or legislation of related laws and regulations will be helpful and needed. These applications will bring new ideas and important changes in different areas like medicine, health and livestock improvement. Especially, the production of bioreactors, drug production and organ culture for human transplantation will provide important economic and social benefits to the human [3].

Other concerns about this subject are the ethical and environmental aspects of transgenesis. The union of an external gene into the genome might disturb the internal gene expression either in the first generation or in the obtained homozygote transgenic animals. In the past, some gene transfer studies have resulted with affected or sick animals and these experiments were finished. If genetic manipulation affect the animal negatively and lead to the suffering, it cannot be accepted by the scientists, the public or regulatory agencies. The environmental issues, including food and ecosystem contamination or threats to biodiversity, are generally seen in transgenic plants than animals. The transgenic animals are used mainly in biomedical applications and therefore do not affect the food chain. It is almost impossible that transgenic domestic animals will go to the wild and mating with feral species. On the basis of biodiversity, the scientists are giving an attention to the traditional genetic selection procedures to preserve some rare breeds. Actually, adding a transgene to a population increases biodiversity, unless the transgenic lines replace other lines with less resistant to a disease. Lastly, social issues and concerns about transgenics have increased and the ethics of using transgenic animals like the health of the genetically modified animal or the safety of these products are getting more attention. Also there are some other social problems remain, for example, a recombinant protein has been obtained from the urine of a transgenic animal may affect the public negatively and block the marketability of that product [31].

Microinjection is the most common used method for the transgenic technology, but sometimes resulted in multi-copies and multi-site integration. In the producing of transgenic fish, the environmental risk is the biggest problem. The result indicates that transgenic fish have lowered fitness compare to the traditional domestic fish. But because of genotype and environment reciprocal effects, it is difficult to determine phenotypes of the transgenic fish [27].

5. Conclusions

Genetic engineering is the process of modifying an organism’s genetic composition by adding foreign genes to produce desired traits or evaluate function. Using genetic engineering, it is possible to constitute transgenic animals. Transgenic animal can be used as a reseach models for production of pharmaceutical animals, disease resistant animals, xenotransplantation, animal cloning and preserving genetic materials for copy of elite animals and to save endangered species.

Presently, environmental disruption and growing population are two main problems in our life. To cover all needs of this growing population is getting more difficult with limited sources. The fast improvement in the biotechnology and growing applications in genetic engineering, especially transgenic technology, getting more attention to solve problems using plants, animals and microorganisms to fulfill the needs. Transgenic technology, provide opportunities in transfer of desired characteristics into different organisms and playing with their structure to meet the demands.

References

[1] D. Jones, Genetic engineering of a mouse: Dr. Frank Ruddle and somatic cell genetics, Yale J. Biol. Med. 84

(2) (2011) 117-124.

[2] D.W. Yu, H.B. Zhu, W.H. Du, Advances of transgenic breeding in livestock, Heredity 33 (5) (2011) 459-468.

[3] X. Miao, Recent advances in the development of new transgenic animal technology, Cell. Mol. Life Sci. 70 (5) (2013) 815-828.

[4] L. Pray, Recombinant DNA technology and transgenic animals: GloFish are the first transgenic animals available to the American public. But what's the biotechnology behind them?, Nature Education 1 (1) (2008) 51.

[5] R.D. Bremel, H.C. Yom, G.T. Bleck, Alteration of milk composition using molecular genetics, J. Dairy Sci. 72 (1989) 2826-2833.

[6] M.B. Wheeler, E.M. Walters, S.G. Clark, Transgenic animals in biomedicine and agriculture: Outlook for the future, Anim. Reprod. Sci. 79 (3-4) (2003) 265-289.

[7] M. Buy, Transgenic Animals, From CCAC Resource Supplement, Spring/Summer, Used with permission of CCAC (Canadian Council on Animal Care), 1997, http://people.ucalgary.ca/~browder/transgenic.html.

[8] J. Xu, N. Li, Biosafety assessment of genetically engineered animals: A review, Journal of Biological Engineering 28 (3) (2012) 267-281. (in Chinese)

[9] T. Sakuma, M.A. Barry, Y. Ikeda, Lentiviral vectors: Basic to translational, Biochem. J. 443 (3) (2012) 603-618.

[10] B. Wu, L. Zan, Enhance beef cattle improvement by embryo biotechnologies, Reprod. Domest. Anim. 47 (5) (2012) 865-871.

[11] B. Aigner, S. Renner, B. Kessler, N. Klymiuk, M. Kurome, A. Wünsch, et al., Transgenic pigs as models for translational biomedical research, J. Mol. Med. (Berl.) 88 (7) (2010) 653-664.

[12] M. Dosay-Akbulut, The benefits of the biotechnological studies in the agriculture and the future of these researches, Minerva Biotechnologica 15 (2003) 173-179.

[13] W.A. Kues, H. Niemann, Advances in farm animal transgenesis, Prev. Vet. Med. 102 (2) (2011) 146-156.

[14] N. Bernardes, R. Seruca, A.M. Chakrabarty, A.M. Fialho, Microbial-based therapy of cancer: Current progress and future prospects, Bioeng. Bugs. 1 (3) (2010) 178-190.

[15] M. de Jesus, F.M. Wurm, Manufacturing recombinant proteins in kg-ton quantities using animal cells in bioreactors, Eur. J. Pharm. Biopharm. 78 (2) (2011) 184-188.

[16] K. Nishijima, S. Iijima, Transgenic chickens, Dev. Growth Differ. 55 (1) (2013) 207-216.

[17] K. Isobe, Z. Cheng, S. Ito, N. Nishio, Aging in the mouse and perspectives of rejuvenation through induced pluripotent stem cells (iPSCs), Results Probl. Cell. Differ. 55 (2012) 413-427.

[18] E.A. Maga, Genetically engineered livestock: Closer than we think? Trends Biotechnol. 23 (11) (2005) 533-535.

[19] T.M. Wilkie, R.D. Palmiter, Analysis of the integrant in Myk-103 transgenic mico in which males fail to transmit the integrant, Mol. Cell. Biol. 7 (1987) 1646-1655.

[20] M. Samiec, M. Skrzyszowska, Transgenic mammalian species, generated by somatic cell cloning, in biomedicine, biopharmaceutical industry and human nutrition/dietetics: Recent achievements, Pol. J. Vet. Sci. 14 (2) (2011) 317-328.

[21] A. Cebrian-Serrano, T. Stout, A. Dinnyes, Veterinary applications of induced pluripotent stem cells: Regenerative medicine and models for disease?, Vet J. 10 (2013) 146-149.

[22] N. Fan, L. Lai, Genetically modified pig models for human diseases, J. Genet. Genomics 40 (2) (2013) 67-73.

[23] H. Chung, T.Y. Kim, S.Y. Lee, Recent advances in production of recombinant spider silk proteins, Curr. Opin. Biotechnol. 23 (6) (2012) 957-964.

[24] K. Jörgens, J.L. Hillebrands, H.P. Hammes, J. Kroll, Zebrafish: A model for understanding diabetic complications, Exp. Clin. Endocrinol. Diabetes 120 (4) (2012) 186-187.

[25] S. Santana, E.P. Rico, J.S. Burgos, Can zebrafish be used as animal model to study Alzheimer's disease?, Am. J. Neurodegener. Dis. 1 (1) (2012) 32-48.

[26] C.L. Hew, P.L. Davies, G. Fletcher, Antifreeze protein gene transfer in Atlantic salmon, Mol. Mar. Biol. Biotechnol. 1 (4-5) (1992) 309-317.

[27] X. Ye, Y.Y. Tian, F.Y. Gao, Progress in transgenic fish techniques and application, Heredity 33 (5) (2011) 494-503.

[28] L.M. Houdebine, Applications of genetically modified animals, J. Soc. Biol. 203 (4) (2009) 323-328.

[29] L.E. Piper, B.M. Bindon, G.H. Davis, The single inheritance of the high litter size of the Borolla Merino, in: R.B. Land, D.W. Robinson (Eds.), Genetics of Reproduction in Sheep, Butterworths, London, 1985, pp. 115-125.

[30] B.B. Scott, T.A. Velho, S. Sim, C. Lois, Applications of avian transgenesis, ILAR J. 51 (4) (2010) 353-361.

[31] M.K. Dyck, D. Lacroix, F. Pothier, M.A. Sirard, Making recombinant proteins in animals: Different systems, different applications, Trends Biotechnol. 21 (9) (2003) 394-399.

Journal of Agricultural Science and Technology A
Volume 4, Number 2, February 2014
David Publishing Company
240 Nagle Avenue #15C, New York, NY 10034, USA
Tel: 1-323-984-7526, 323-410-1082; Fax: 1-323-984-7374, 323-908-0457
http://www.davidpublishing.com, www.davidpublishing.org
agriculture@davidpublishing.com, agriculture@davidpublishing.org

ISSN 2161-6256